NEW TRANSFECTION REAGENTS

This application is a continuation of co-pending U.S. Patent Application No. 09/438,365, filed November 12, 1999, which claims priority from U.S. Provisional Patent Application No. 60/108,117, filed November 12, 1998. To the extent that they are consistent herewith, the aforementioned applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates to cationic lipids and compositions of cationic lipids having utility in lipid aggregates for delivery of macromolecules and other compounds into cells.

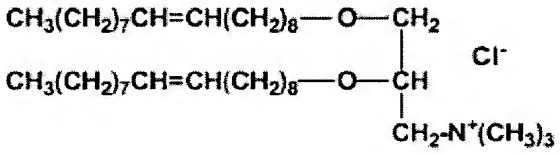
Related Art

Lipid aggregates such as liposomes have been found to be useful as agents for delivery to introduce macromolecules, such as DNA, RNA, protein, and small chemical compounds such as pharmaceuticals, to cells. In particular, lipid aggregates comprising cationic lipid components have been shown to be especially effective for delivering anionic molecules to cells. In part, the effectiveness of cationic lipids is thought to result from enhanced affinity for cells, many of which bear a net negative charge. Also in part, the net positive charge on lipid aggregates comprising a cationic lipid enables the aggregate to bind polyanions, such as nucleic acids. Lipid aggregates containing DNA are known to be effective agents for efficient transfection of target cells.

The structure of various types of lipid aggregates varies, depending on composition and method of forming the aggregate. Such aggregates include liposomes, unilamellar vesicles, multilameller vesicles, micelles and the like, having particular sizes in the nanometer to micrometer range. Methods of making lipid aggregates are by now well-known in the art. The main drawback to use of conventional phospholipid containing liposomes for delivery is that the material to be delivered must be encapsulated and the liposome composition has a net negative charge which is not attracted to the negatively charged cell surface. By combining cationic lipid compounds with a phospholipid, positively charged vesicles and other types of lipid aggregates can bind DNA, which is negatively charged, can be taken up by target cells, and can transfect target cells. (Felgner, P.L. et al.

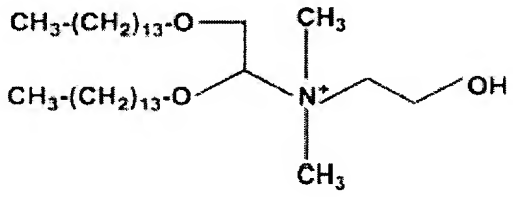
(1987) Proc. Natl. Acad. Sci. USA 84:7413-7417; Eppstein, D. et al., U.S. Pat. No. 4,897,355.)

A well-known cationic lipid is N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA). The structure of DOTMA is:

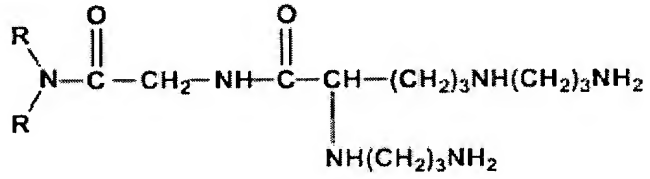


DOTMA by itself or in 1:1 combination with dioleoylphosphatidylethanolamine (DOPE) is formulated into liposomes using standard techniques. Felgner, et al. supra demonstrated that such liposomes provided efficient delivery of nucleic acids to some types of cells. A DOTMA:DOPE (1:1) formulation is sold under the trade name LIPOFECTIN (Life Technologies, Inc., Rockville, MD). Another commercially available cationic lipid is 1,2-bis(oleoyloxy)-3-3-(trimethylammonia) propane (DOTAP), which differs from DOTMA only in that the oleoyl moieties are linked via ester, rather than ether bonds to the propylamine. A related group of compounds differ from DOTMA and DOTAP in that one of the methyl groups of the trimethylammonium group is replaced by a hydroxyethyl group. Compounds of this type are similar to the Rosenthal Inhibitor (RI) of phospholipase A (Rosenthal, A.F. and Geyer, R.P. (1960) J. Biol. Chem. 235:2202-2206) which has stearoyl esters linked to the propylamine core. The dioleoyl analogs of RI are commonly abbreviated as DORI-ether and DORI-ester, depending on the linkage of the fatty acid moieties to the propylamine core. The hydroxy group can be used as a site for further functionalization.

The dimyristyloxy analog of RI is known as DRMIE. A 1:1 (M/M) DMRIE:cholesterol formulation is sold under the tradename DMRIE-C (Life Technologies, Inc., Rockvilee, MD). The structure of DMRIE is:

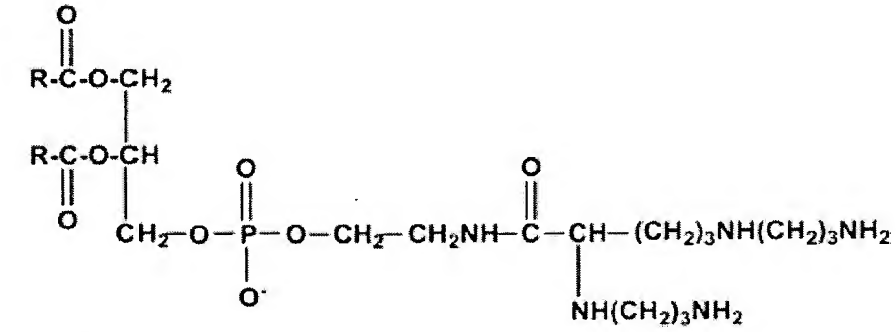


Another class of compounds has been disclosed by Behr et al. (1989) Proc. Natl. Acad. Sci. USA 86:6982-6986; EPO publication 0 394 111 (Oct. 24, 1990), in which carboxyspermine has been conjugated to two types of lipids. The structures of 5-carboxyspermylglycine dioctadecylamide (DOGS) is:



$R=CH_3(CH_2)_{17}$

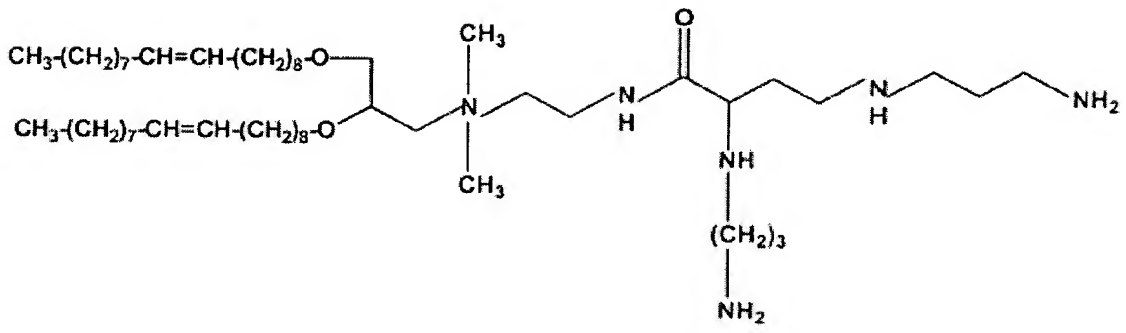
The structure of dipalmitoylphosphatidylethanolamine 5-carboxyspermylamide (DPPES) is:



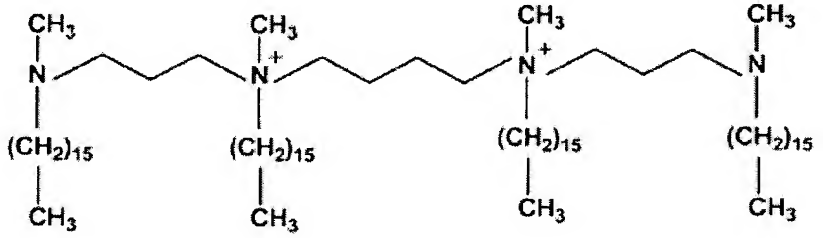
$R=CH_3(CH_2)_{15}$

Both DOGS and DPPES have been used to coat plasmids, forming a lipid aggregate complex that provides efficient transfection. The compounds are claimed to be more efficient and less toxic than DOTMA for transfection of some cell lines. DOGS is available commercially as TRANSFECTAM ™ (Promega, Madison, Wis.).

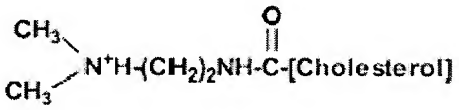
Another class of compounds has been also described in which carboxy spermine has been conjugated to lipids via an amide bond (Gebeyehu, G. et al., U.S. Patent No. 5,334,761). These compounds are useful for an efficient delivery of nucleic acids into various cells and also are intermediates for making other such lipids. 2,3-di-oleyloxy-N-[2(spermine-carboxamido)ethyl]-N,N-dimethyl-l-propan-aminium (DOSP A) is available as a 3:1 (w/w) formulation with DOPE under the trade name LipofectAMINE (available from Life Technologies, Inc., Rockville, MD). The structure of DOSPA is as follows:



Lipid compounds with a spermine head group have also been described (Haces, A., et al., U.S. Patent No. 5,674,908). These compounds are especially useful for delivery of nucleic acids into insect cells. A 1: 1.5 (M/M) formulation of tetramethyltetrapalmityl spennine (TM- TPS) to DOPE is commercially available under the tradename CellFECTIN (Life Technologies, mc., Rockville, MD). The structure of TM- TPS is shown below:



A cationic cholesterol derivative (DC-Chol) has been synthesized and formulated into liposomes in combination with DOPE. (Gao. X. and Huang, L. (1991) Biochim. Res. Cornrn. 179:280-285). The compound's structure is:



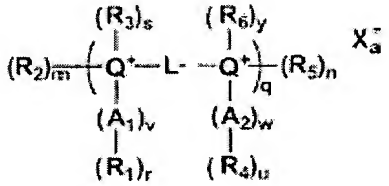
Liposomes formulated with DC-Chol are said to provide more efficient transfection and lower toxicity than DOTMA-containing liposomes for some cell lines.

Lipopolylysine, formed by conjugating polylysine to DOPE, has been reported to be especially effective for transfection in the presence of serum, a condition likely to be encountered in vivo (Zhou, X. et al. (1991) Biochim. Biophys. Acta 1065: 8-14).

Despite advances in the field, a need remains for a variety of improved cationic lipid compounds. In particular, no single cationic lipid to date has been found to work well with all cell types. Since different cell types differ from one another in membrane composition, it is not surprising that different compositions and types of lipid aggregates are effective for different cell types, either for their ability to contact and fuse with target cell membranes, or for aspects of the transfer process itself. At present these processes are not well understood, consequently the design of effective liposomal precursors is largely empirical. Besides content and transfer, other factors are of importance, for example, ability to form lipid aggregates suited to the intended purpose, the possibility of transfecting cells in the presence of serum, toxicity to the target cell, stability as a carrier for the compound to be delivered, and ability to function in an *in vivo* environment. In addition, lipid aggregates can be improved by broadening the range of substances which can be delivered to cells. The cationic lipid compounds of the present invention have improved function with respect to several of the foregoing attributes.

SUMMARY OF THE INVENTION

The present invention provides novel cationic lipids according to the general Formula (A):



wherein

Q is selected from the group consisting of N, O and S;

L is any bivalent organic radical capable of covalently linking each Q, such as C, CH, $(CH_2)l$ or $\{(CH_2)i$ -Y -$(CH_2)j\}k$, wherein Y is selected from the group consisting of CH_2, an ether, a polyether, an amide, a polyamide, an ester, a sulfide, a urea, a thiourea, a guanidyl, a carbamoyl, a carbonate, a phosphate, a sulfate, a sulfoxide, an imine, a carbonyl, and a secondary amino group and wherein Y is optionally substituted by $-X_1$-L '-X_2-Z or -Z;

R_1-R_6, independently of one another, are selected from the group consisting of

H, -{CH_2)$_p$-D-Z, an alkyl, an alkenyl, an aryl, and an alkyl or alkyl ether optionally substituted by one or more of an alcohol, an amino alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, an alkylthio, a urea, a thiourea, a guanidyl, or a carbamoyl group, and wherein at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group; and anyone or more of R_1, R_3, R_4 and R_6 may optionally be covalently linked with each other, with Y or with L when L is C or CH to form a cyclic moiety;

Z is selected from the group consisting of amine, spermiyl, carboxyspermiyl, guanidyl, spermidinyl, putricinyl, diaminoalkyl, pyridyl, piperidinyl, pyrrolidinyl, polyamine, amino acid, peptide, and protein;

X_1 and X_2, independently of one another, are selected from the group consisting of NH, O, S, alkylene, and arylene;

L' is selected from the group consisting of alkylene, alkenylene, alkynylene, arylene, alkylene ether, and polyether;

D is Q or a bond;

A_1 and A_2, independently of one another, are selected from the group consisting of CH_2O, CH_2S, CH_2NH, C(O), C(NH), C(S) and $(CH_2)t$;

X is a physiologically acceptable anion;

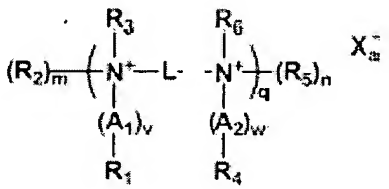
m, n, r, s, u, v, w and y are 0 or 1, with the proviso that when both m and n are 0 at least one of r, s, u and y is other than 0;

i, j, k, 1, p and t are from a to about 100;

q is an integer from 1 to about 1000; and

a is the number of positive charge divided by the valence of the anion.

Further, the present invention provides novel cationic lipids according to the general Formula (B):



wherein

L is $(CH_2)l$ or $\{(CH_2)i$ -Y- $(CH_2)j\}k$ wherein Y is selected from the group consisting of an ether, a polyether, an amide, a polyamide, an ester, a sulfide, a urea, a thiourea, a guanidyl, a carbamoyl, a carbonate, and a secondary amino group;

R_l –R_6, independently of one another, are selected from the group consisting of H, $-(CH_2)_p$-Z, an alkyl, an alkenyl, an aryl, and an alkyl or an alkyl ether optionally substituted by one or more of an alcohol, an aminoalcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group, and at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group, preferably having from about 2 to 100, preferably 4 to 75, more preferably 6 to 64, more preferably 8 to 50, more preferably 8 to 40, more preferably 8 to 30, more preferably 6 to 30, more preferably 4 to 30, more preferably 2 to 30, and most preferably 8 to about 24 carbon atoms, and anyone or more of R_1, R_3, R_4 and/or R_6 may optionally be covalently linked with each other to form a cyclic moiety;

Z is selected from the group consisting of amine, spemliyl, carboxyspemliyl, guanidyl, spemlidinyl, putricinyl, diaminoalkyl, pyridyl, piperidinyl, pyrrolidinyl, polyamine, amino acid, amino acid derivative, peptide, and protein;

A_l and A_2, independently of one another, are selected from the group consisting of CH_2O, CH_2S, CH_2NH, C(O), C(NH), C(S) and $(CH_2)t$;

X is a physiologically acceptable anion, such as the halide anions, chloride, bromide, and iodide as well as acetate, sulfate, trifluoroacetate, etc.;

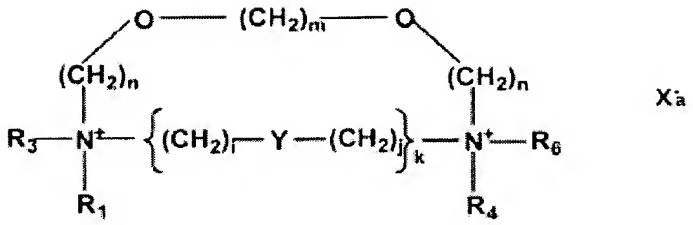
m, n, v and w are 0 or 1;

i, j, k, 1, p and t are integers from 1 to about 100, more preferably 1 to 50, more preferably 1 to 25, more preferably 1 to 15, more preferably 1 to 10 and most preferably 1 to about 4;

q is an integer from 1 to about 1000, preferably from 1 to about 500, more preferably from 1 to about 250, more preferably from 1 to about 100, more preferably from 1 to about 50, more preferably from 1 to about 25, more preferably from 1 to about 12, most preferably from 1 to about 6; and

a is the number of positive charges divided by the valence of the anion, wherein when m and n are 0, then a is O.

Also, the present invention provides novel cationic lipids according to the Formula (C):



wherein

Y is selected from the group consisting of CH_2, an ether, a polyether, an amide, a polyamide, an ester, a sulfide, a urea, a thiourea, a guanidyl, a carbamoyl, a carbonate, a phosphate, a sulfate, a sulfoxide, an imine, a carbonyl, and a secondary amino group and wherein Y is optionally substituted by $-X_1-L'-X_2-Z$ or -Z;

R_1, R_3, R_4 and R_6, independently of one another, are selected from the group consisting of H, $-\{CH_2\}_p-D-Z$, an alkyl, an alkenyl, an aryl, and an alkyl or an alkyl ether optionally substituted by one or more of an alcohol, an aminoalcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, an alkylthio, a urea, a thiourea, a guanidyl, or a carbamoyl group, and at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group, most preferably having from about 8 to about 24 carbon atoms, and R_1, R_3, R_4 and R_6 may optionally be covalently linked with each other or with Y, to form a cyclic moiety;

Z is selected from the group consisting of amine, spenniyl, caboxyspenniyl, guanidyl, spennidinyl, putricinyl, diaminoalkyl, pyridyl, piperidinyl, pyrrolidinyl, polyamine, amino acid, peptide, and protein;

X_1 and X_2, independently of one another, are selected from the group consisting of NH, O, S, alkylene, and arylene;

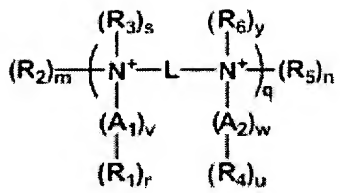
L' is selected from the group consisting of alkylene, alkenylene, alkynylene, arylene, alkylene ether, and polyether;

D is Q or a bond;

m and n are 0 or 1; and

i, j, k, l and p are integers from 1 to about 10.

Further, the present invention provides compounds or polycations according to the Formula (D):



wherein

L is C, CH, (CH_2)l or {(CH2)i -Y -(CH_2)j}k, wherein Y is selected from the group consisting of CH_2, an ether, a polyether, an amide, a polyamide, an ester, a sulfide, a urea, a thiourea, a guanidyl, a carbamoyl, a carbonate, a phosphate, a sulfate, a sulfoxide, an imine, a carbonyl, and a secondary amino group and wherein Y is optionally substituted by -X_1-L '-X_2-Z or -Z;

R_1 –R_6, independently of one another, are selected from the group consisting of H, -$(CH_2)_p$-D-Z, an alkyl, an alkenyl, an aryl, and an alkyl or an alkyl ether optionally substituted by one or more of an alcohol, an aminoalcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, an alkylthio, a urea, a thiourea, a guanidyl, or a carbamoyl group, and wherein at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl groups, preferably having from about 2 to about 30 carbon atoms, more preferably from 8 to 24 carbon atoms;

Z is selected from the group consisting of amine, spenniyl, carboxyspenniyl, guanidyl, spennidinyl, putricinyl, diaminoalkyl, pyridyl, piperidinyl, pyrrolidinyl, polyamine, amino acid, amino acid derivative, peptide, and protein;

X_1 and X_2, independently of one another, are selected from the group consisting of NH, O, S, alkylene and arylene;

L' is selected from the group consisting of alkyl ene, alkenylene, alkynylene, arylene, alkylene ether, and polyether;

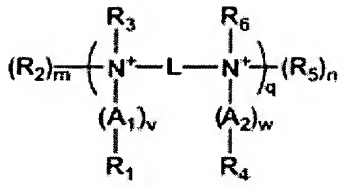
A_1 and A_2, independently of one another, are selected from the group consisting of CH_2O, CH_2S, CH_2NH, C(O), C{NH), C(S) and (CH_2)t;

m, n, r, s, u, v, w and y are 0 or 1, with the proviso that when both m and n are 0 at least one of r, s, u and y is other than 0;

i, j, k, 1, p and t are integers from 0 to about 100; and

q is an integer from 1 to about 1000.

Also, the present invention provides compounds or polycations according to the Formula (E):



wherein

L is $(CH_2)l$ or $\{(CH_2)i$ -Y- $(CH_2)j\}k$, wherein Y is selected from the group consisting of CH2, an ether, a polyether, an amide, a polyamide, an ester, a sulfide, a urea, a thiourea, a guanidyl, a carbamoyl, a carbonate, and a secondary amino group;

$R_1 - R_6$, independently of one another, are selected from the group consisting of H, -$(CH_2)p$-Z, an alkyl, an alkenyl, an aryl, and an alkyl or an alkyl ether optionally substituted by one or more of an alcohol, an amino alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group, and at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkenyl or aryl group, preferably having from about 2 to about 30 carbon atoms, more preferably having from about 8 to about 24 carbon atoms;

Z is selected from the group consisting of amine, spenniyl, carboxyspenniyl, guanidyl, spennidinyl, putricinyl, diaminoalkyl, pyridyl, piperidinyl, pyrrolidinyl, polyamine, amino acid, amino acid derivative, peptide, and protein;

A_1 and A_2, independently of one another, are selected from the group consisting of CH2O, CH2S, CH2NH, C(O), C(NH), C(S) and (CH2)t;

m, n, v and w are 0 or 1;

i, j, k, 1, p and t are integers from 1 to about 100; and

q is an integer from 1 to about 1000.

Also, the present invention provides novel compounds falling within the scope of the above formulae.

The compounds of the invention are useful, either alone or in combination with other lipid aggregate-forming components (e.g., DOPE, DOPC or cholesterol) for formulation into liposomes or other lipid aggregates. Such aggregates are polycationic, able to form stable complexes with anionic macromolecules, such as nucleic acids. The lipid aggregate macromolecular complex interacts with cells making the polyanionic macromolecule available for absorption and uptake by the cell.

The present invention provides a lipid aggregate comprising one or more of the compounds of the present invention. Preferably, the lipid aggregate comprises at least one lipid aggregate-forming compound. Preferably, the lipid aggregate-forming compound is selected from the group consisting of DOPE, DOPC and cholesterol.

The compounds of the present invention may also be conjugated to or mixed with or used in conjunction with a variety of useful molecules and substances such as proteins, peptides, growth factors and the like to enhance cell-targeting, uptake, internalization, nuclear targeting and expression.

This invention also includes lipid aggregates comprising one or more compounds of the present invention or mixtures thereof. Such lipid aggregates may be combined with one or more aggregate-forming components and/or transfection enhancers.

The transfection methods of the present invention employing the compounds or compositions (such as those described above) of the present invention or mixtures thereof can be applied to in vitro and in vivo transfection of cells, particularly to transfection of eukaryotic cells or tissues including animal cells, human cells, insect cells, plant cells, avian cells, fish cells, mammalian cells and the like.

Accordingly, the present invention provides a method for introducing a polyanion into a cell or cells, wherein the method comprises forming a liposome from a positively charged compound according to the invention, contacting the liposome with polyanion to form a positively-charged polyanion-liposome complex and incubating the complex with a cell or cells.

The methods of this invention can be used to generate transfected cells or tissues which express useful gene products. The methods of this invention can also be used as a step in the production of transgenic animals. The methods of this invention are useful in any therapeutic method requiring introducing of nucleic acids into cells or tissues. In particular, these methods are useful in cancer treatment, in *in vivo* and *ex vivo* gene therapy, and in diagnostic methods. See, for example, U.S. Patent No. 5,589,466 to

Felgner, et al. and U.S. Patent Application No. 08/450,555 filed on May 25, 1995 to Jessee, et al. The transfection compounds or compositions of this invention can be employed as research reagents in any transfection of cells or tissues done for research purposes. Nucleic acids that can be transfected by the methods of this invention include DNA and RNA from any source comprising natural bases or non-natural bases, and include those encoding and capable of expressing therapeutic or otherwise useful proteins in cells or tissues, those which inhibit expression of nucleic acids in cells or tissues, those which inhibit enzymatic activity or activate enzymes, those which catalyze reactions (ribozymes), and those which function in diagnostic assays.

The compounds, compositions and methods provided herein can also be readily adapted in view of the disclosure herein to introduce biologically active macromolecules or substances other than nucleic acids, including, among others, polyamines, polyamine acids, polypeptides, proteins, biotin, and polysaccharides into cells. Other useful materials for example, therapeutic agents, diagnostic materials and research reagents, can be introduced into cells by the methods of this invention. In a preferred aspect, any nucleic acid vector may be delivered to or into a cell by the present invention.

Accordingly, the present invention provides a method for introducing a biologically active substance into a cell, wherein the method comprises forming a liposome of a compound according to the invention and a biologically active substance and incubating the liposome with a cell or cell culture.

The invention also relates to compositions comprising the compounds of the invention and one or more additional components selected from the group consisting of nucleic acids, cells, buffers, culture media, biologically active substance, neutral lipids, and transfection enhancers, preferably a nucleic acid.

This invention also includes transfection kits which include one or more of the compounds or compositions of the present invention or mixtures thereof. Particularly, the invention provides a kit comprising one or more of the compounds of the present invention and at least one additional component selected from the group consisting of a cell, cells, a cell culture media, a nucleic acid, a transfection enhancer and instructions for transfecting a cell or cells.

The invention also relates to intermediates and methods for using such intermediates for making the compounds or compositions of the invention. The invention also relates to the compositions, compounds or components obtained by the interaction of materials (intermediates, compounds, lipids etc.) used in the synthesis methods of the invention.

Other preferred embodiments of the present invention will be apparent to one of ordinary skill in the art in view of the following drawings and description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the transfection of HEK-293 cells with cationic transfection reagents.

Figure 2 is a graph showing transfection of COS- 7 cells with cationic transfection reagents.

Figure 3 is a graph showing transfection of CHO-KI cells with cationic transfection reagents.

Figure 4 is a graph showing transfection of He La cells with cationic transfection reagents.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

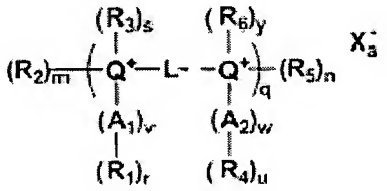
The present invention relates to cationic lipids and compositions of cationic lipids having utility in lipid aggregates for delivery of macromolecules and other compounds into cells. The compounds can be used alone or in combination with other compounds to prepare liposomes and other lipid aggregates suitable for transfection or delivery of compounds to target cells, either in vitro or in vivo.

The compounds of the present invention are preferably polycationic and preferably thus form highly stable complexes with various anionic macromolecules, particularly polyanions such as nucleic acids. These compounds have the property, when dispersed in water, of forming lipid aggregates which associate strongly, via their cationic portion, with polyanions. By using an excess of cationic charges relative to the anionic compound, the polyanion-lipid complexes may be adsorbed on cell membranes, thereby facilitating uptake of the desired compound by the cells.

The present invention also relates to intermediates for preparing the compound and compositions of the invention.

More specifically, the present invention relates to a cationic lipid for transfection which has a greater transfection efficiency than commercially available products in the three most common cell types used in expression research (CHO-K1, COS- 7, and HEK293) making it useful for high throughput applications; and which has a simple to use protocol as defined by the fact that no additional reagents are required (e.g., such as LipofectAMINE PLUS Reagent available from Life Technologies, Inc., Rockville, MD), no removal of serum and therefore no media changes are required, and the DNA/lipid complex do not need to be removed from the cells prior to assay.

The compounds according to the present invention have the Formula (A):



wherein

Q is selected from the group consisting of N, O and S;

L is any bivalent organic radical capable of covalently linking each Q, such as C, CH, (CH_2)l or {(CH_2)i -Y -(CH_2)j}k, wherein Y is selected from the group consisting of CH_2, an ether, a polyether, an amide, a polyamide, an ester, a sulfide, a urea, a thiourea, a guanidyl, a carbamoyl, a carbonate, a phosphate, a sulfate, a sulfoxide, an imine, a carbonyl, and a secondary amino group and wherein Y is optionally substituted by -X1-L '-X2-Z or -Z;

$R_1 - R_6$, independently of one another, are selected from the group consisting of H, -(CH_2)p-D-Z, an alkyl, an alkenyl, an aryl, and an alkyl or alkyl ether optionally substituted by one or more of an alcohol, an amino alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, an alkylthio, a urea, a thiourea, a guanidyl, or a carbamoyl group, and wherein at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group; and R_1 and R_4 or R_3 and R_6 may optionally be covalently linked with each other, with Y or with L when L is C or CH to form a cyclic moiety;

Z is selected from the group consisting of amine, spermiyl, carboxyspermiyl, guanidyl, spermidinyl, putricinyl, diaminoalkyl, pyridyl, piperidinyl, pyrrolidinyl, polyamine, amino acid, peptide, and protein;

X_1 and X_2, independently of one another, are selected from the group consisting of NH, O, S, alkylene, and arylene;

L' is selected from the group consisting of alkylene, alkenylene, alkynylene, arylene, alkylene ether, and polyether;

D is Q or a bond;

A_1 and A_2, independently of one another, are selected from the group consisting of CH_2O, CH_2S, CH_2NH, C(O), C(NH), C(S) and $(CH_2)t$;

X is a physiologically acceptable anion;

m, n, r, s, u, v, w and y are 0 or 1, with the proviso that when both m and n are 0 at least one of r, s, u and y is other than 0;

i, j, k, l, p and t are integers from 0 to about 100;

q is an integer from 1 to about 1000; and

a is the number of positive charge divided by the valence of the anion.

Preferably the alkyl ether optionally substituted by one or more alcohol groups comprises a carbohydrate. Preferably, the carbohydrate is selected from the group consisting of galactose, fructose, glucose, maltose, sucrose, cellobiose, lactose, mannose, glucopyranose, mannopyranose and galactopyranose.

Preferably, i, j, k, 1, p and t are integers independently selected from 1 to 100, more preferably from 1 to 50, more preferably 1 to 25, more preferably 1 to 15, more preferably 1 to 10 and most preferably 1 to about 4. Preferably, 1, b and c are integers from 1 to about 4, i and j are integers from about 2 to about 3 and k is an integer from 1 to about 3.

Preferably, q is an integer from 1 to about 500, more preferably from 1 to about 250, more preferably from 1 to about 100, more preferably from 1 to about 50, more preferably from 1 to about 25, more preferably from 1 to about 12, most preferably from 1 to about 6.

Preferably, at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group having from about 2 to 100, preferably 4 to 75, more

preferably 6 to 64, more preferably 8 to 50, more preferably 8 to 40, more preferably 8 to 30, more preferably 6 to 30, more preferably 4 to 30, and most preferably 8 to about 24 carbon atoms.

In all aspects of the invention, most suitable R_1 and R_4 groups, which can be the same or different, preferably the same, are C_{6-30} hydrocarbon radicals derived from fatty acids or activated derivatives thereof, such as fatty acyl chlorides. Thus, typical R_1 and R_4 groups are C_{6-30} alkyl or alkenyl groups.

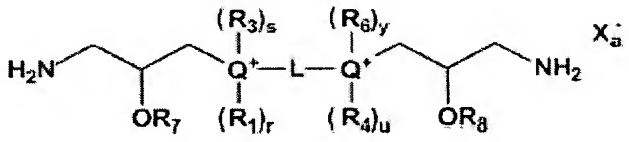
Preferably, R_1, R_2, R_3, R_4, R_5 and R_6, independently of one another, are selected from the group consisting of H, C_1-C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group, and at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group having from about 8 to about 24 carbon atoms.

Preferably Q is N.

Preferably, Y is selected from the group consisting ofCH_2, O, S and NH.

Useful compounds falling within the scope of the above formula (A) include compounds having the following formulae:

(A1)



wherein

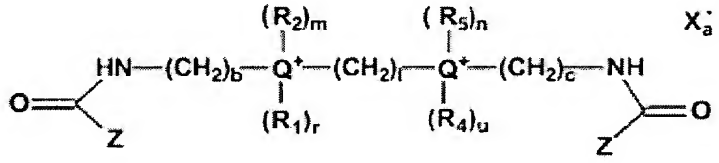
Q and L are as defined above;

R_1, R_3, R_4 and R_6, independently of one another, are selected from the group consisting of H and a C_1-C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

r, s, u and y are 0 or 1; and

R_7 and R_8 are independently H or a carbohydrate;

(A2)



wherein

Q is as defined above;

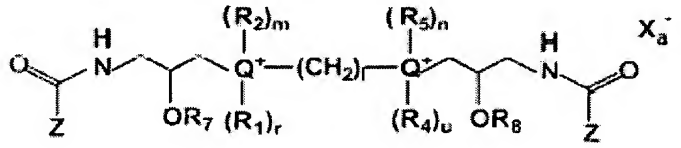
R_1, R_2, R_4 and R_5, independently of one another, are selected from the group consisting of H and a C_1-C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

Z is selected from the group consisting of spermiyl, spermidiyl, amino acid, peptidyl, diaminoalkyl, and polyamine;

m, n, r and u are 0 or l; and

l, b and c are integers independently selected from 1 to about 4;

(A3)



wherein

Q, R_1, R_4, m, n, r and u are as defined above;

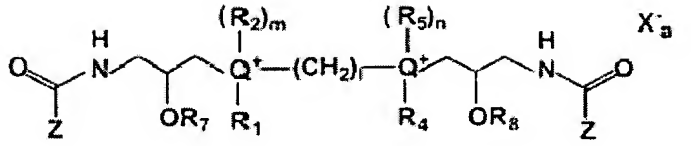
R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1-C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

Z is selected from the group consisting of spermiyl, spermidiyl, amino acid, peptidyl, diaminoalkyl, and polyamine;

R_7 and R_8 are independently H or a carbohydrate; and

l is an integer from 1 to about 4;

(A4)



wherein

Q is as defined above, preferably N;

at least one of R_1 and R_4 are straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl groups having from about 8 to about 24 carbon atoms;

R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1-C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

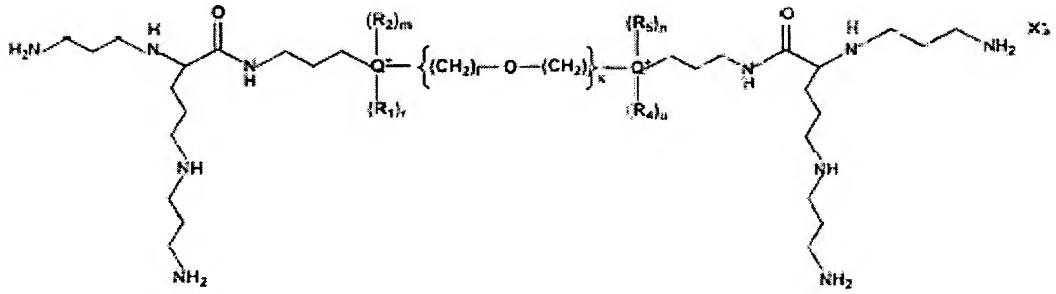
Z is selected from the group consisting of spermiyl, spermidiyl, amino acid, peptidyl, diaminoalkyl, and polyamine;

R_7 and R_8 are independently H or a carbohydrate, preferably H;

m and n are as defined above; and

l is an integer from 1 to about 4;

(A5)



wherein

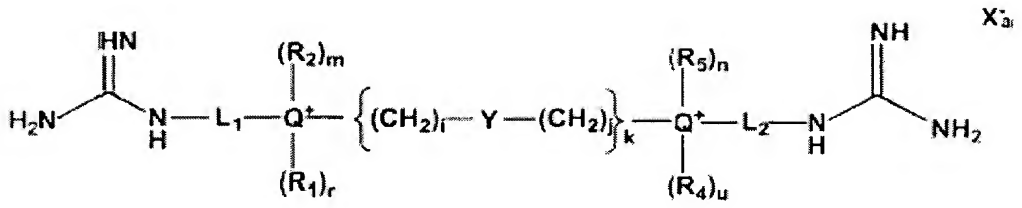
Q, R_1, R_4, r, u, m and n are as defined above;

R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1-C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, apolyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

i and j are integers from about 2 to about 3; and

k is an integer from 1 to about 3;

(A6)



wherein

Q, R_1, R_4, r, u, m and n are as defined above;

R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1 –C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

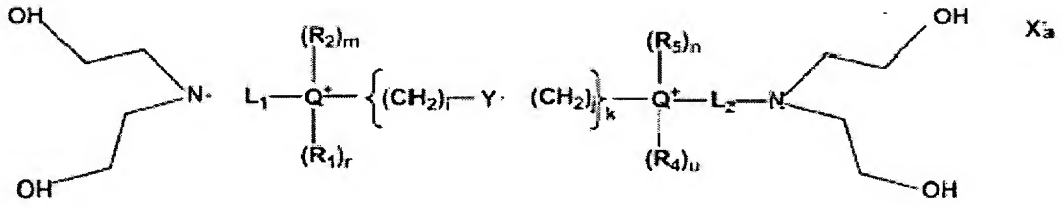
i and j are integers from about 2 to about 3;

k is an integer from 1 to about 3;

L_1 and L_2, independently from one another, are an alkylene or an alkylene ether;

Y is selected from the group consisting of CH_2, O, S and NH;

(A7)



wherein

Q, R_1, R_4, r, u, m and n are as defined above;

R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1 –C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

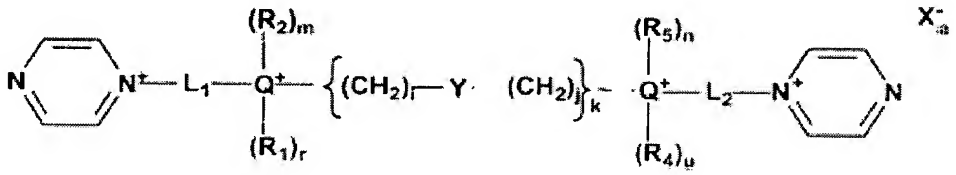
i and j are integers from about 2 to about 3;

k is an integer from 1 to about 3;

L_1 and L_2, independently from one another, are an alkylene or an alkylene ether;

Y is selected from the group consisting of CH_2, O, S and NH;

(A8)



wherein

Q, R_1, R_4, r, u, m and n are as defined above;

R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1 –C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

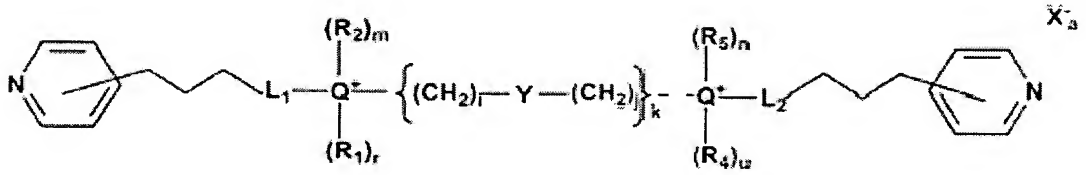
i and j are integers from about 2 to about 3;

k is an integer from 1 to about 3;

L_1 and L_2, independently from one another, are an alkylene or an alkylene ether;

Y is selected from the group consisting of CH_2, O, S and NH;

(A9)



wherein

Q, R_1, R_2, r, u, m and n are as defined above;

R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1 –C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

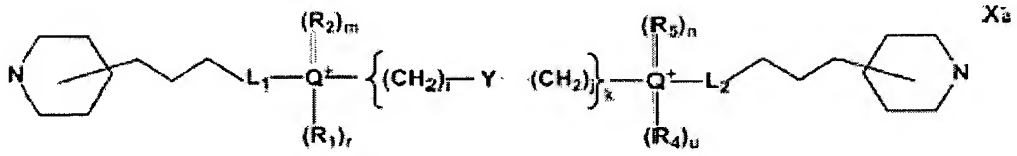
i and j are integers from about 2 to about 3;

k is an integer from 1 to about 3;

L_1 and L_2, independently from one another, are an alkylene or an alkylene ether;

Y is selected from the group consisting of CH_2, O, S and NH; and

(A10)



wherein

Q, R_1, R_4, r, u, m, and n are as defined above;

R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1 –C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

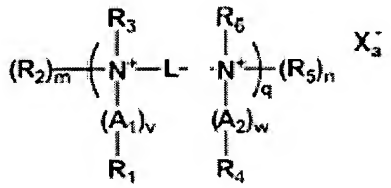
i and j are integers from about 2 to about 3;

k is an integer from 1 to about 3;

L_1 and L_2, independently from one another, are an alkylene or an alkylene ether;

Y is selected from the group consisting of CH_2, O, S and NH.

Also, compounds of the present invention have the Formula (B):



wherein

L is $(CH_2)l$ or $\{(CH_2)i$ -Y -$(CH_2)j\}k$, wherein Y is selected from the group consisting of CH2, an ether, a polyether, an amide, a polyamide, an ester, a sulfide, a urea, a thiourea, a guanidyl, a carbamoyl, a carbonate, and a secondary amino group;

R_1 –R_6, independently of one another, are selected from the group consisting of H, -(CH_2)p-Z, an alkyl, an alkenyl, an aryl, and an alkyl or alkyl ether optionally substituted by one or more of an alcohol, an aminoalcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group, and at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or

aryl group, and anyone or more of R_1, R_4, R_3 and R_6 may optionally be covalently linked with each other to form a cyclic moiety;

Z is selected from the group consisting of amine, spermiyl, carboxyspermiyl, guanidyl, spermidinyl, putricinyl, diaminoalkyl, pyridyl, piperidinyl, pyrrolidinyl, polyamine, amino acid, peptide, and protein;

A_1 and A_2, independently of one another, are selected from the group consisting of CH_2O, CH_2S, CH_2NH, C(O), C(NH), C(S) and $(CH_2)t$;

X is a physiologically acceptable anion;

m, n, v and w are 0 or 1;

i, j, k, 1, p and t are integers from 1 to about 100;

q is an integer from 1 to about 1000; and

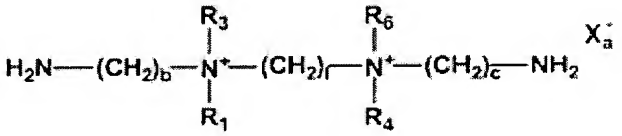
a is the number of positive charge divided by the valence of the anion, wherein when m and n are 0, then a is 0.

Preferably, R_1-R_6, i, j, k, l, p, t, q, b and c are as defined with reference to Formula (A).

Preferably, Y is selected from the group consisting of CH_2, O, S and NH.

Useful compounds falling within the scope of the Formula (B) include compounds having the following formulae:

(B1)

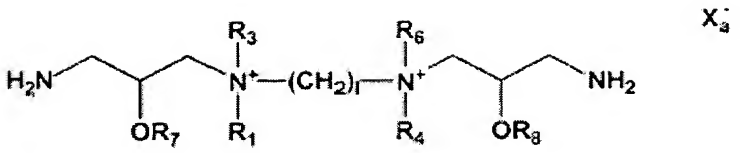


wherein

R_1, R_3, R_4 and R_6, independently of one another, are selected from the group consisting of H and a C_1-C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group, and at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group having from about 8 to about 24 carbon atoms; and

l, b and c are integers independently selected from 1 to about 4;

(B2)



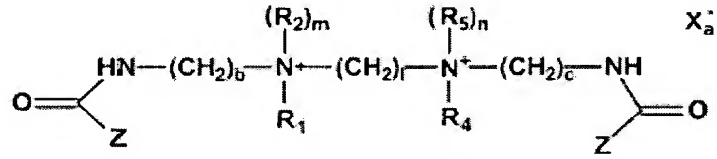
wherein

R_1, R_3, R_4 and R_6, independently of one another, are selected from the group consisting of H and a C_1-C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group, and at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group having from about 8 to about 24 carbon atoms;

R_7 and R_8 are independently H or a carbohydrate; and

l is an integer from 1 to about 4;

(B3)



wherein

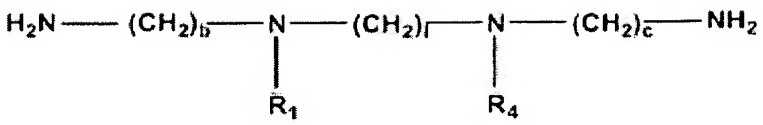
R_1, R_2, R_4 and R_5, independently of one another, are selected from the group consisting of H and a C_1-C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group, and at least one of R_1, R_2, R_3 and R_5 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group having from about 8 to about 24 carbon atoms;

Z is selected from the group consisting of spermiyl, spermidiyl, amino acid, peptidyl, diaminoalkyl, and polyamine;

m and n are 0 or 1; and

l, b and c are integers independently selected from 1 to about 4;

(B4)

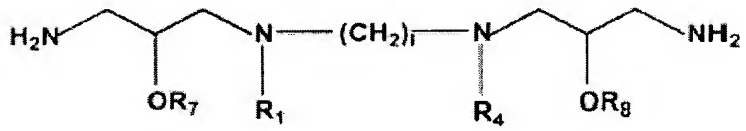


wherein

at least one of R_1 and R_4 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group having from about 8 to about 24 carbon atoms; and

l, b and c are integers independently selected from 1 to about 4;

(B5)

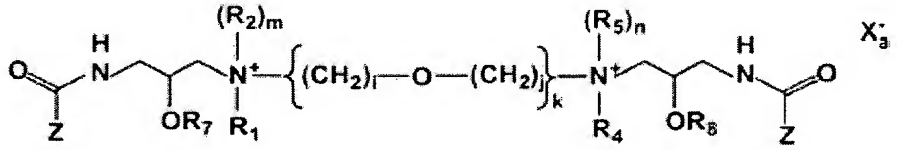


wherein

at least one of R_1 and R_4 are straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl groups having from about 8 to about 24 carbon atoms; R_7 and R_8 are independently hydrogen or a carbohydrate, preferably hydrogen; and

l is an integer from 1 to about 4;

(B6)



wherein

Z is as defined above;

at least one of R_1 and R_4 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group having from about 8 to about 24 carbon atoms;

R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1-C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

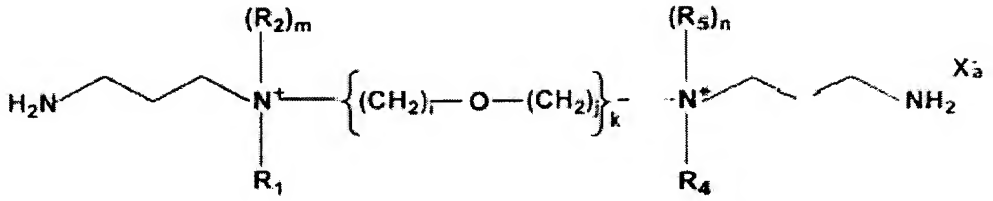
R_7 and R_8 are independently H or a carbohydrate;

m and n are 0 or 1;

i and j are integers from about 2 to about 3; and

k is an integer from 1 to about 3;

(B7)



wherein

at least one of R_1 and R_4 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group having from about 8 to about 24 carbon atoms;

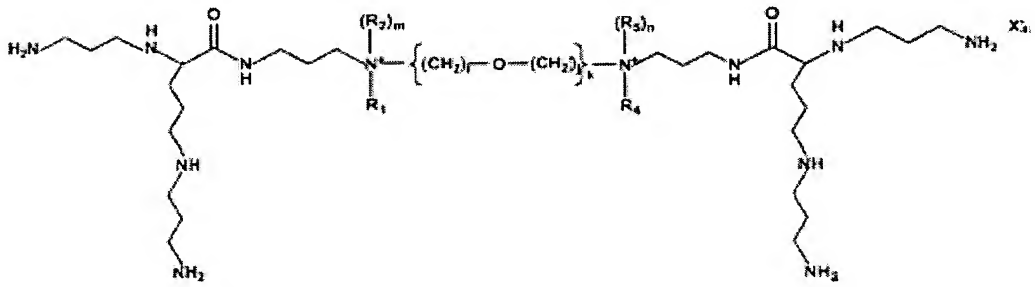
R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1-C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

m and n are 0 or 1;

i and j are integers from about 2 to about 3; and

k is an integer from 1 to about 3;

(B8)



wherein

at least one of R_1 and R_4 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group having from about 8 to about 24 carbon atoms;

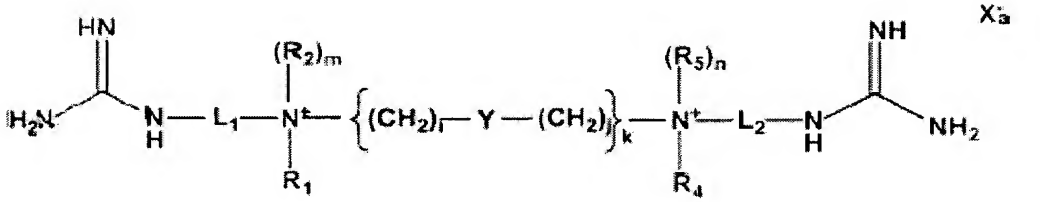
R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1-C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

m and n are 0 or 1;

i and j are integers from about 2 to about 3; and

k is an integer from 1 to about 3;

(B9)



wherein

at least one of R_1 and R_4 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group having from about 8 to about 24 carbon atoms;

R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1 –C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

m and n are 0 or 1;

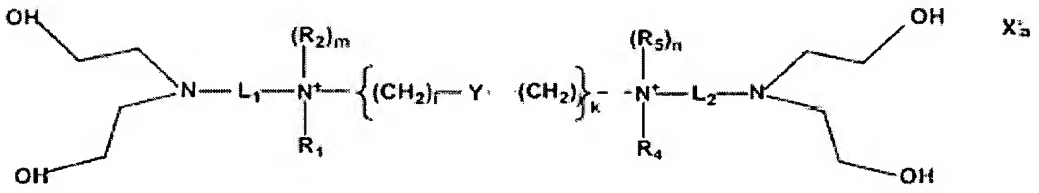
i and j are integers from about 2 to about 3;

k is an integer from 1 to about 3;

L_1 and L_2, independently from one another, are an alkylene or an alkylene ether;

Y is selected from the group consisting of CH_2, O, S and NH;

(B10)



wherein

at least one of R_1 and R_4 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group having from about 8 to about 24 carbon atoms;

R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1-C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol,

an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

m and n are 0 or 1;

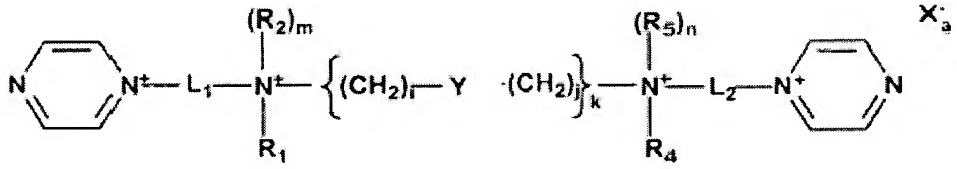
i and j are integers from about 2 to about 3;

k is an integer from 1 to about 3;

L_1 and L_2, independently from one another, are an alkylene or an alkylene ether;

Y is selected from the group consisting of CH_2, O, S and NH;

(B11)



wherein

at least one of R_1 and R_4 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group having from about 8 to about 24 carbon atoms;

R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1 –C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

m and n are 0 or 1;

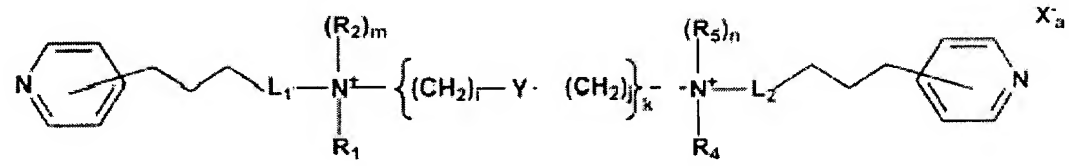
i and j are integers from about 2 to about 3;

k is an integer from 1 to about 3;

L_1 and L_2, independently from one another, are an alkylene or an alkylene ether;

Y is selected from the group consisting of CH_2, O, S and NH;

(B12)



wherein

at least one of R_1 and R_4 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group having from about 8 to about 24 carbon atoms;

R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1 –C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

m and n are 0 or 1;

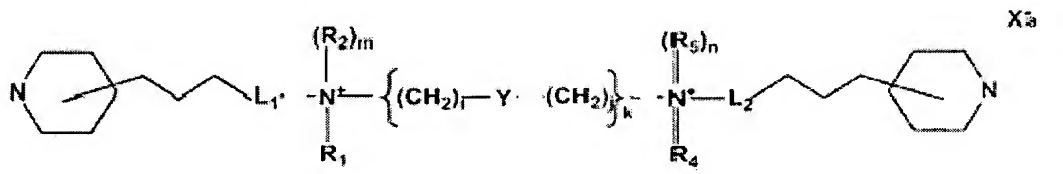
i and j are integers from about 2 to about 3;

k is an integer from 1 to about 3;

L_1 and L_2, independently from one another, are an alkylene or an alkylene ether;

Y is selected from the group consisting of CH_2, O, S and NH; and

(B13)



wherein

at least one of R_1 and R_4 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group having from about 8 to about 24 carbon atoms;

R_2 and R_5, independently of one another, are selected from the group consisting of H and a C_1 –C_8 alkyl, alkenyl, aryl, and alkyl optionally substituted by one or more of an alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group;

m and n are 0 or 1;

i and j are integers from about 2 to about 3;

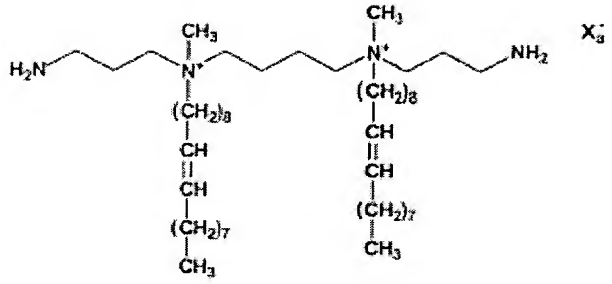
k is an integer from 1 to about 3;

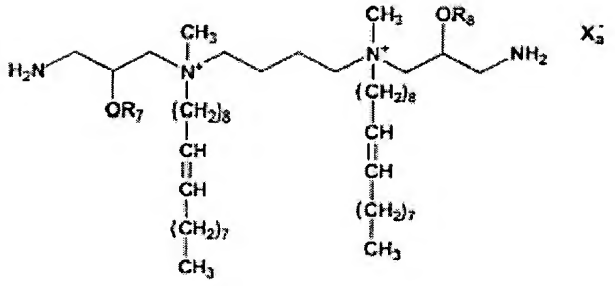
L_1 and L_2, independently from one another, are an alkylene or an alkylene ether;

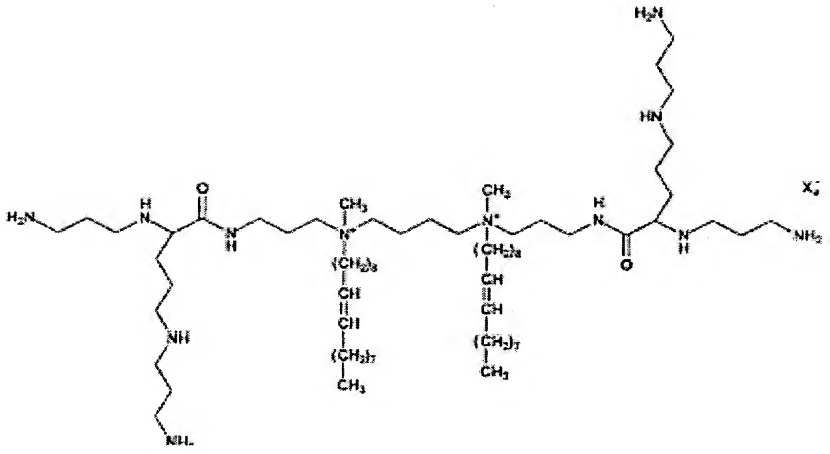
Y is selected from the group consisting of CH_2, O, S and NH.

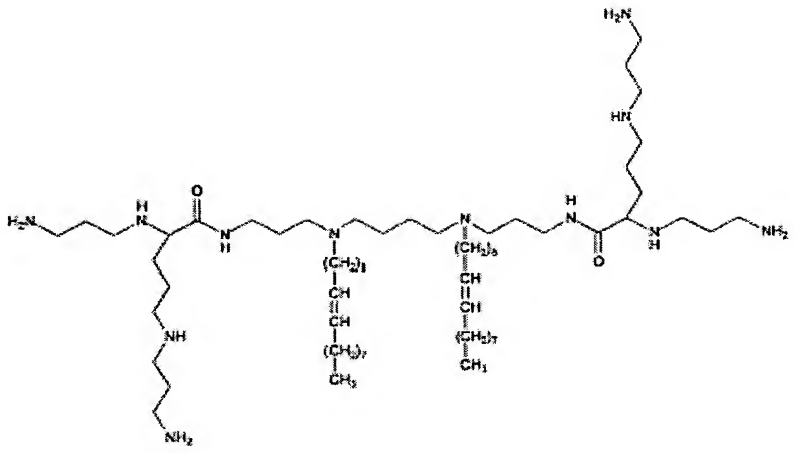
In each of formulae (B1) through (B13) preferably R_1 and R_4 are each C_{6-30} alkyl or alkenyl, more preferably C_{8-24} alkyl or alkenyl, and R_2 and R_5 or R_3 and R_6 are each hydrogen or C_{1-8} alkyl.

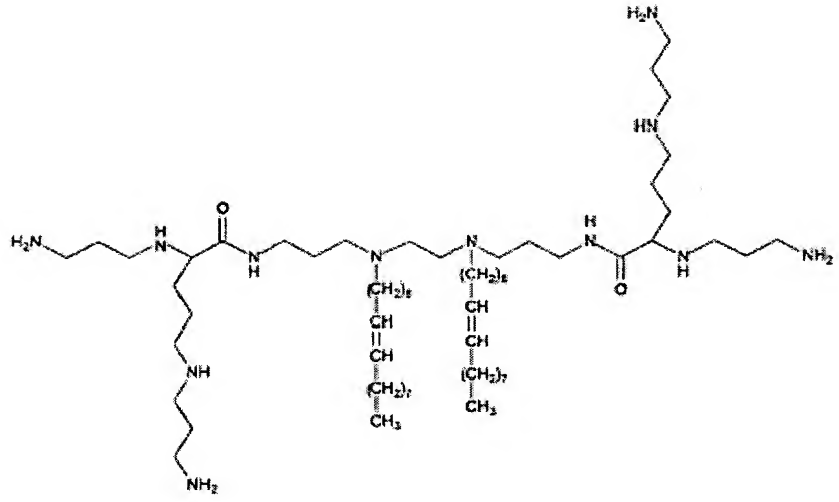
Specific compounds within the scope of the invention include the following examples. R_7 and R_8 in the formulae are independently H or a carbohydrate, preferably H.

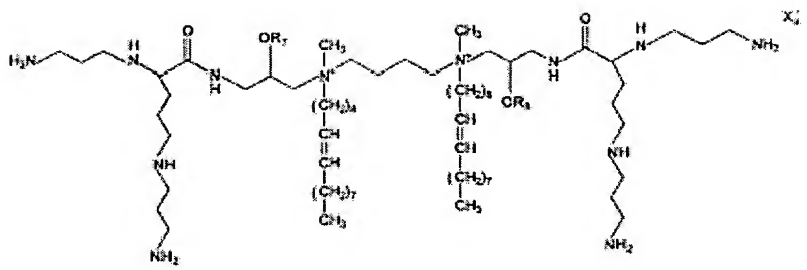


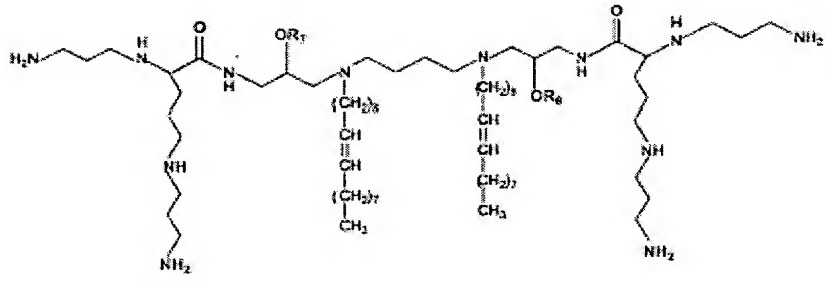


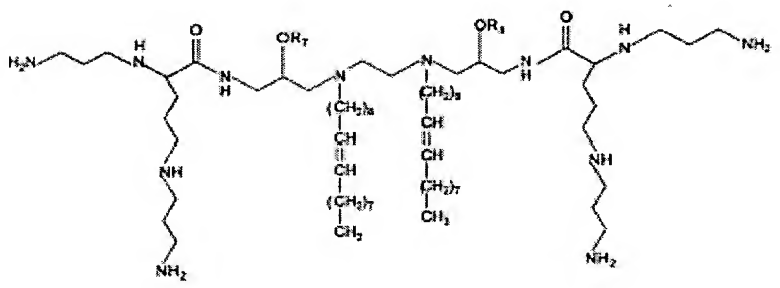


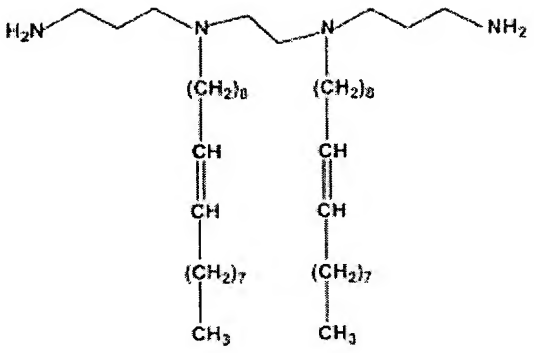


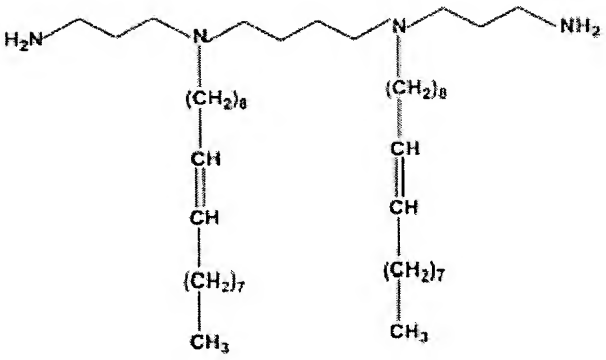


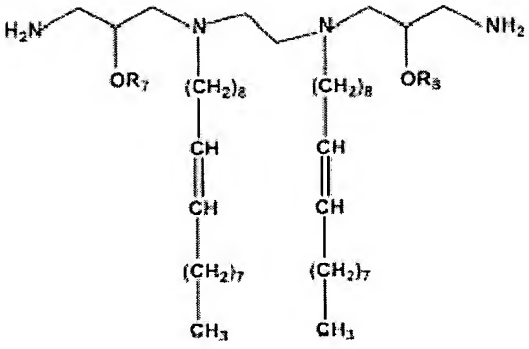


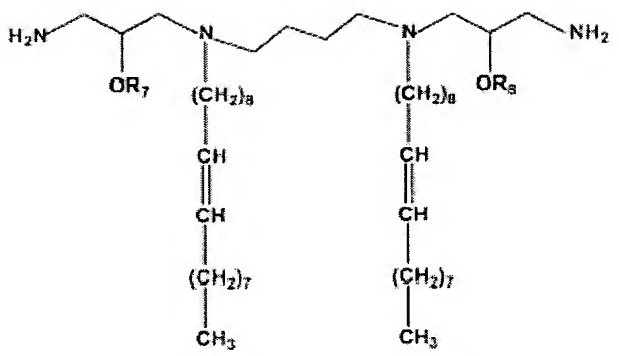


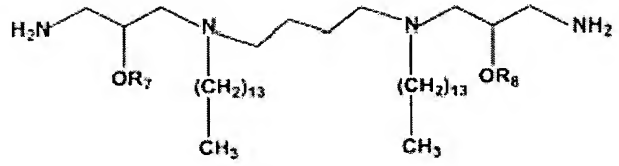


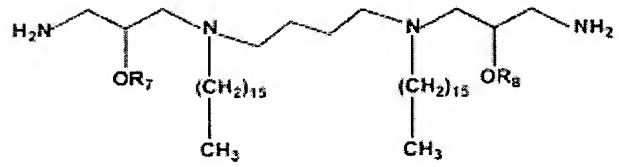












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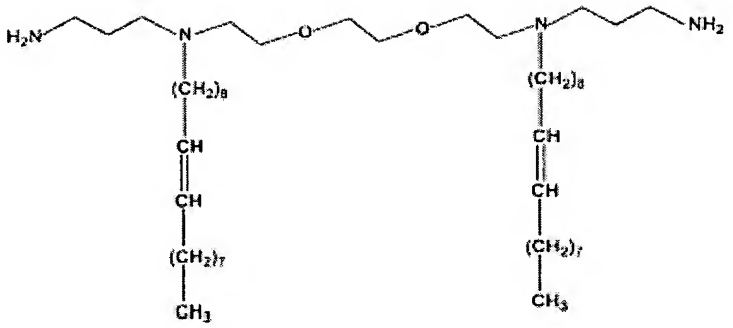

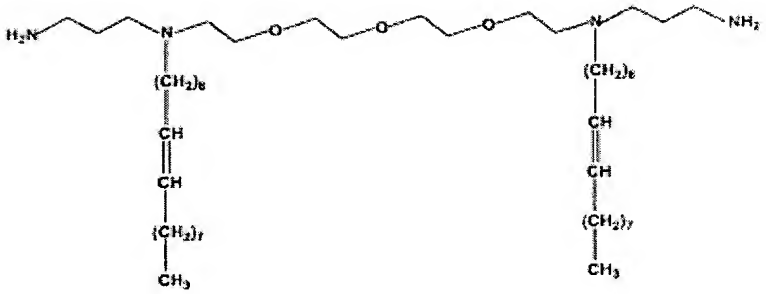

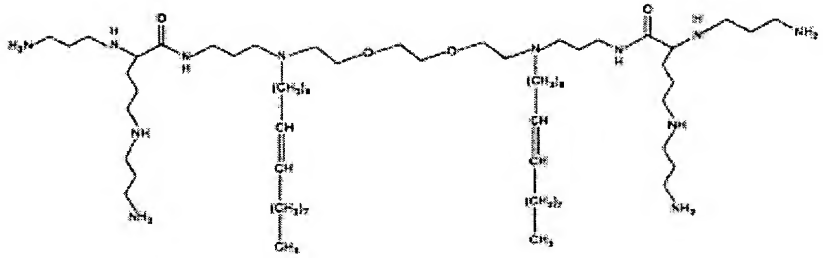

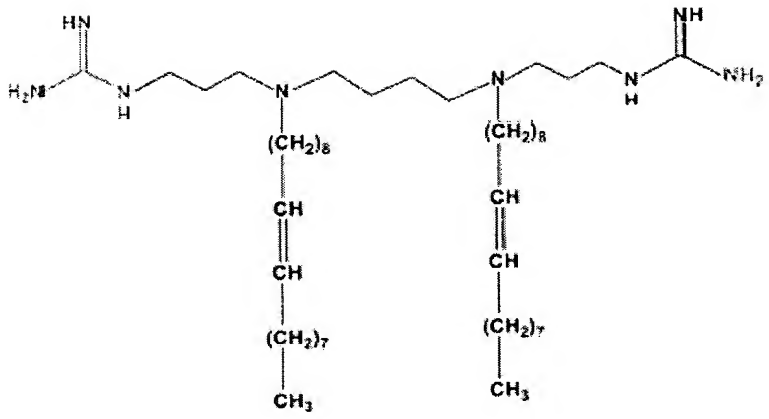

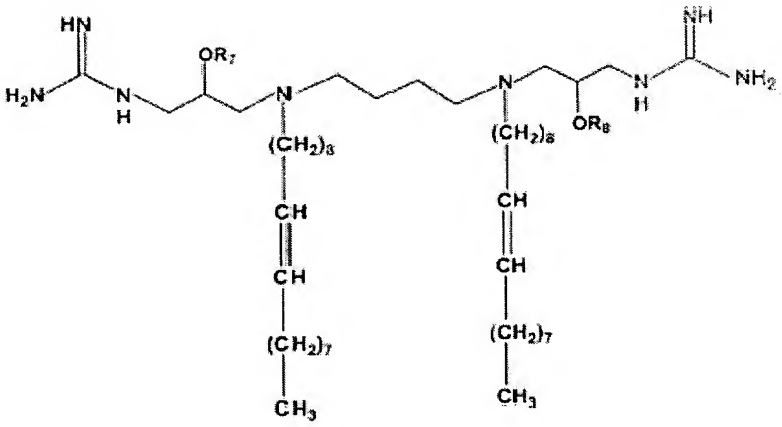

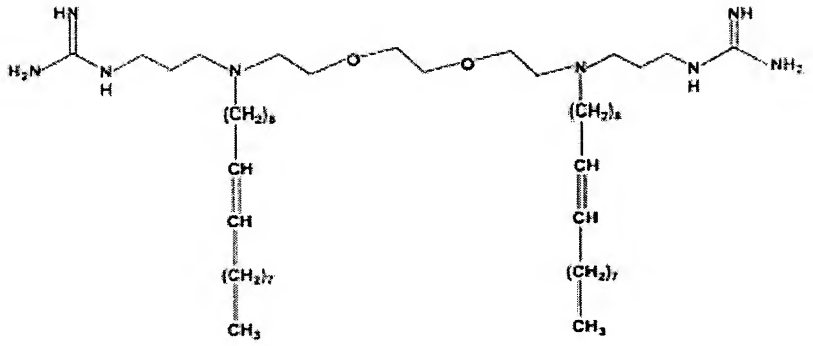

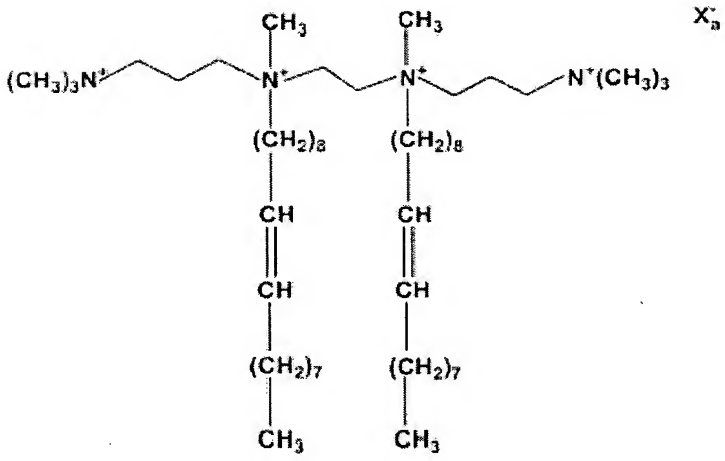

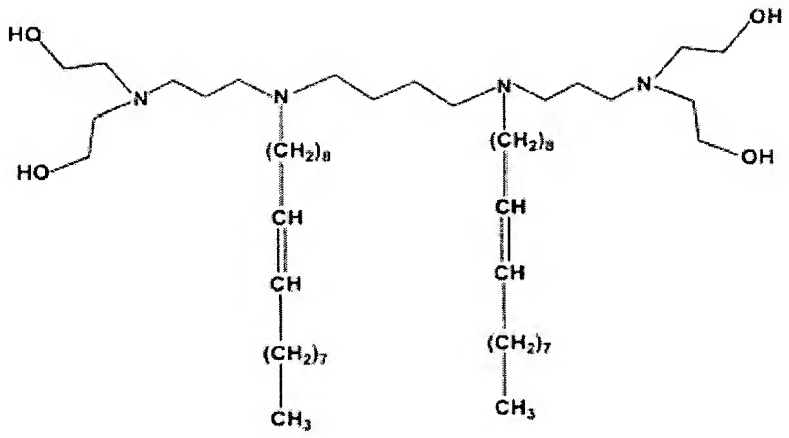

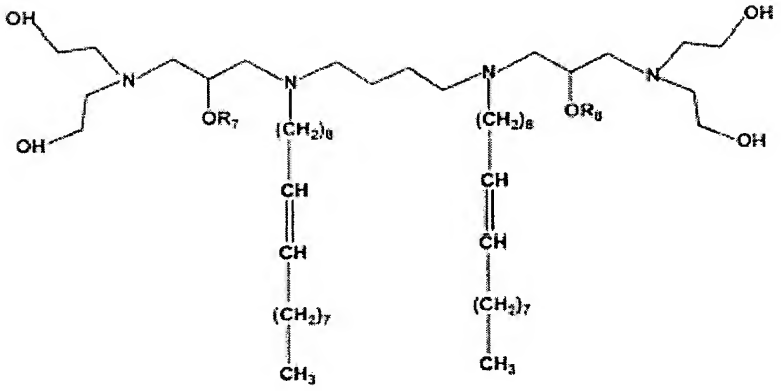

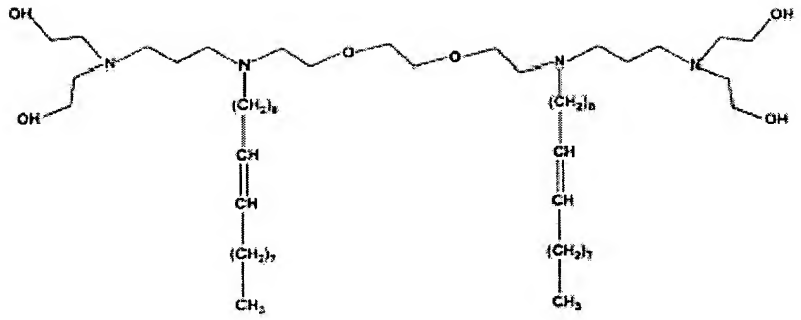

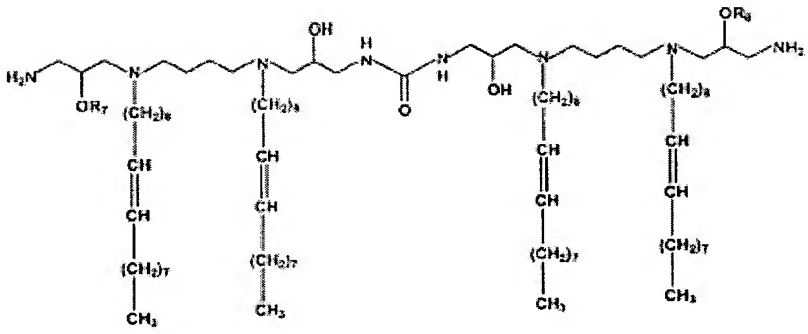

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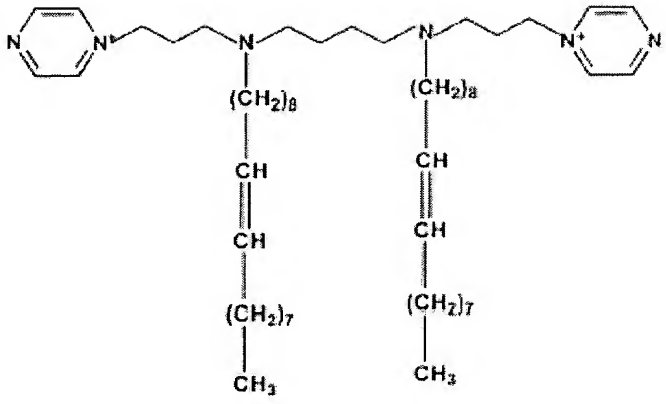

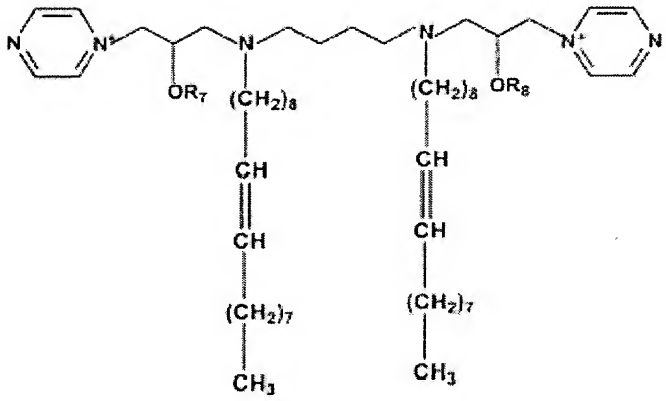

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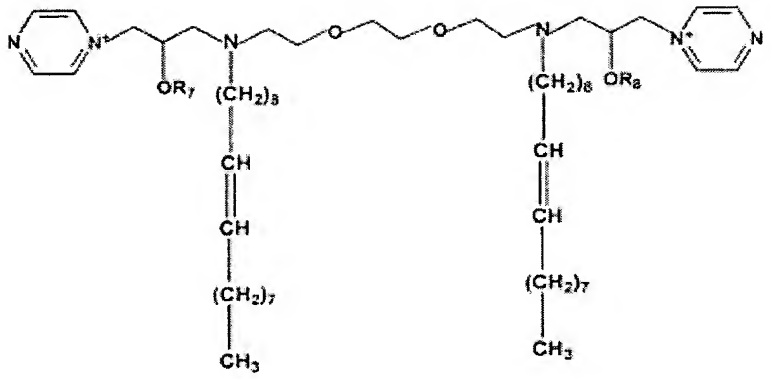
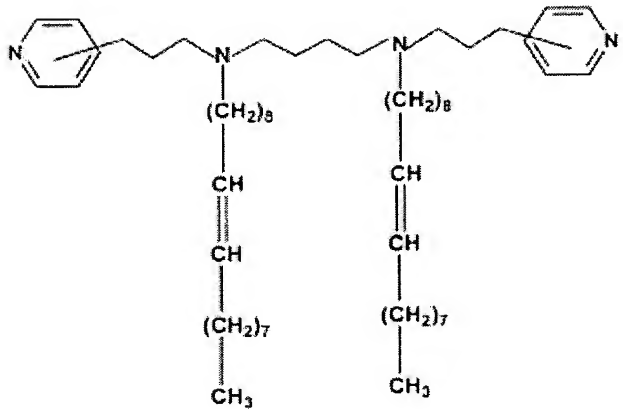
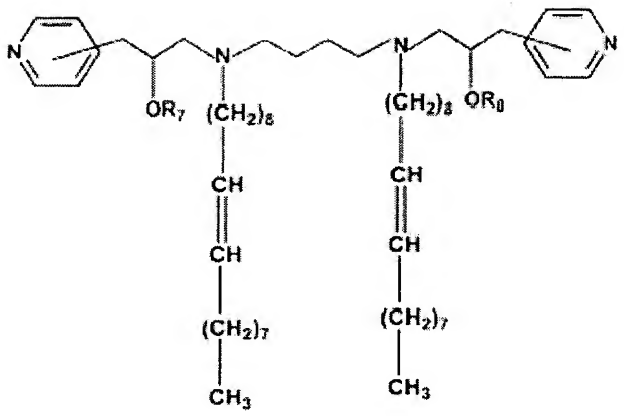
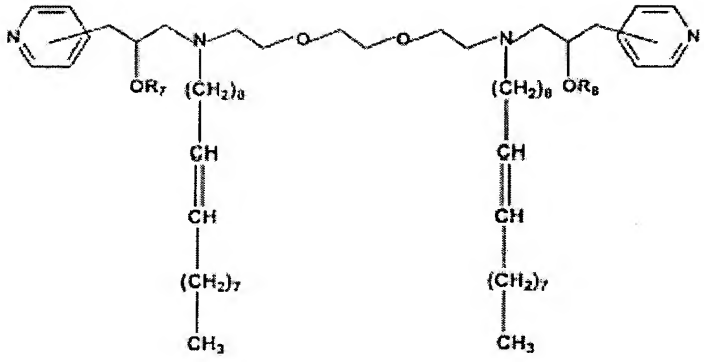
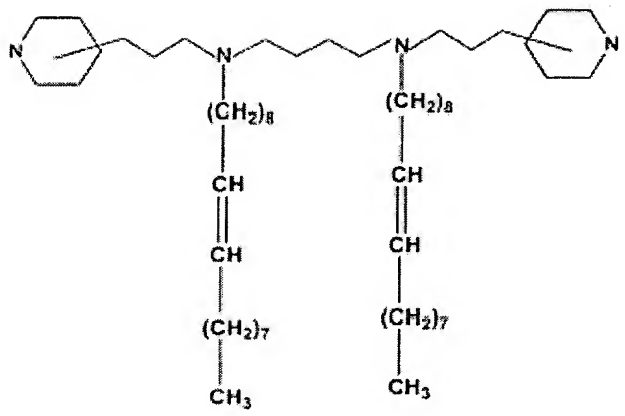
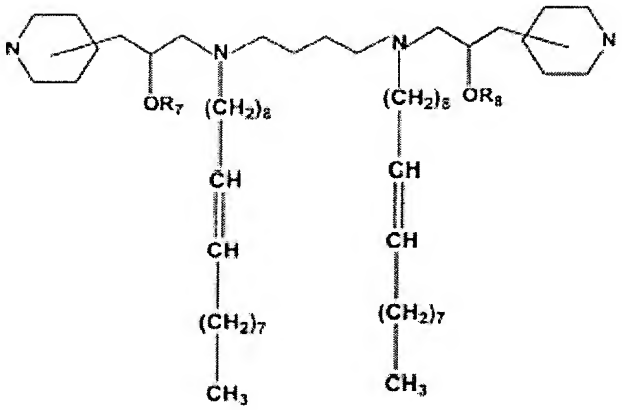


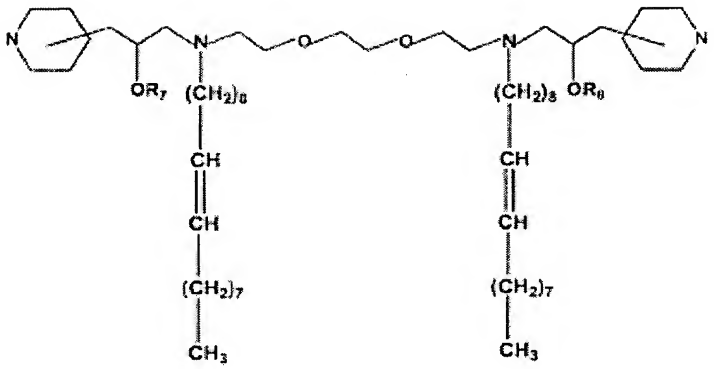


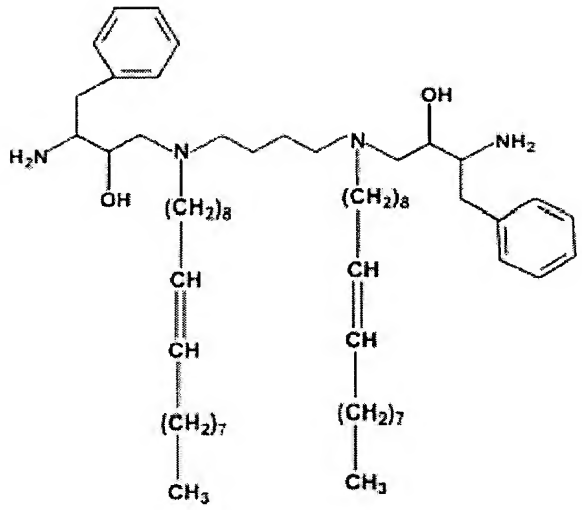




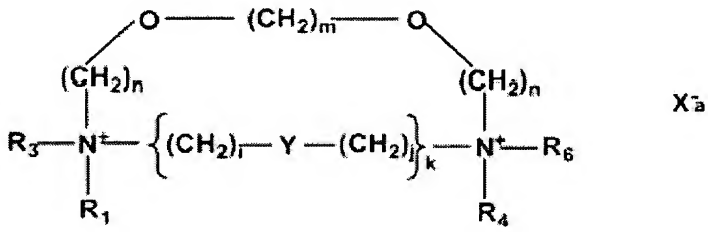








Further, the compounds according to the present invention have the Formula (C):



wherein

Y is selected from the group consisting of CH_2, an ether, a polyether, an amide, a polyamide, an ester, a sulfide, a urea, a thiourea, a guanidyl, a carbamoyl, a carbonate, a phosphate, a sulfate, a sulfoxide, an imine, a carbonyl, and a secondary amino group and wherein Y is optionally substituted by $-X_1$-L '-X_2-Z or -Z;

R_1, R_3, R_4 and R_6, independently of one another, are selected from the group consisting of H, -(CH_2)p-D-Z, an alkyl, an alkenyl, an aryl, and an alkyl or an alkyl ether optionally substituted by one or more of an alcohol, an aminoalcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, an alkylthio, a urea, a thiourea, a guanidyl, or a carbamoyl group, and at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group, and R_1, R_3, R_4 and R_6 may optionally be covalently linked with each other or with Y, to form a cyclic moiety;

Z is selected from the group consisting of amine, spermiyl, carboxyspermiyl, guanidyl, spermidinyl, putricinyl, diaminoalkyl, pyridyl, piperidinyl, pyrrolidinyl, polyamine, amino acid, peptide, and protein;

X_1 and X_2, independently of one another, are selected from the group consisting of NH, O, S, alkylene, and arylene;

L' is selected from the group consisting of alkylene, alkenylene, alkynylene, arylene, alkylene ether, and polyether;

D is Q or a bond;

m and n are 0 or 1; and

i, j, k, I and p are integers independently selected from 1 to about 10.

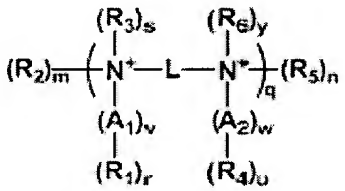
Preferably, Y is selected from the group consisting of CH_2, an ether, a polyether, an amide, a polyamide, an ester, a sulfide, a urea, a thiourea, a guanidyl, a carbamoyl, a carbonate, and a secondary amino group.

Preferably, R_1, R_3, R_4 and R_6, independently of one another, are selected from the group consisting of H, -(CH_2)p -Z, an alkyl, an alkenyl, an aryl, and an alkyl or an alkyl ether optionally substituted by one or more of an alcohol, an aminoalcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group, and at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkenyl or aryl group, and R_1, R_3, R_4 and R_6 may be covalently linked with each other, to form a cyclic moiety.

Preferably, at least one of R_1 and R_4 is straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group having from about 2 to 100, preferably 4 to 75, more preferably 6 to 64, more preferably 8 to 50, more preferably 8 to 40, more preferably 8 to 30, more preferably 6 to 30, more preferably 4 to 30, and most preferably 8 to about 24 carbon atoms.

Preferably, Y is selected from the group consisting of CH_2, O, S and NH.

The compounds and polycations of the present invention have the following Formula (D):



wherein

L is C, CH, (CH_2)l, or {(CH_2)i -Y -(CH_2)j}k, wherein Y is selected from the group consisting of CH2, an ether, a polyether, an amide, a polyamide, an ester, a sulfide, a urea, a thiourea, a guanidyl, a carbamoyl, a carbonate, a phosphate, a sulfate, a sulfoxide, an imine, a carbonyl, and a secondary amino group and wherein Y is optionally substituted by $-X_1$-L '-X_2 -Z or -Z.

$R_1 - R_6$, independently of one another, are selected from the group consisting of H, -(CH_2)p-D-Z, an alkyl, an alkenyl, an aryl, and an alkyl or an alkyl ether optionally substituted by one or more of an alcohol, an amino alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, an alkylthio, a urea, a thiourea, a guanidyl, or a carbamoyl group, and wherein at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group;

Z is selected from the group consisting of amine, spermiyl, carboxyspermiyl, guanidyl, spermidinyl, putricinyl, diaminoalkyl, pyridyl, piperidinyl, pyrrolidinyl, polyamine, amino acid, amino acid derivative, peptide, and protein;

X_1 and X_2, independently of one another, are selected from the group consisting of NH, O, S, alkylene and arylene;

L' is selected from the group consisting of alkylene, alkenylene, alkynylene, arylene, alkylene ether, and polyether;

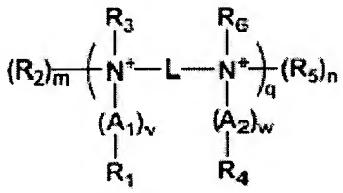
A_1 and A_2, independently of one another, are selected from the group consisting of CH_2O, CH_2S, CH_2NH, C(O), C(NH), C(S) and $(CH_2)t$;

m, n, r, s, u, v, w and y are 0 or 1, with the proviso that when both m and n are 0 at least one of r, s, u and y is other than 0;

i, j, k, 1, p and t are integers from 0 to about 100; and

q is an integer from 1 to about 1000.

Also, the compounds and the polycations of the present invention have the following Formula (E):



wherein

L is $(CH_2)l$ or $\{(CH_2)i$ -Y- $(CH_2)j\}k$, wherein Y is selected from the group consisting of CH_2, an ether, a polyether, an amide, a polyamide, an ester, a sulfide, a urea, a thiourea, a guanidyl, a carbamoyl, a carbonate, and a secondary amino group;

$R_1 - R_6$, independently of one another, are selected from the group consisting of H, $-(CH_2)p-Z$, an alkyl, an alkenyl, an aryl, and an alkyl or an alkyl ether optionally substituted by one or more of an alcohol, an amino alcohol, an amine, an amide, an ether, a polyether, a polyamide, an ester, a mercaptan, a urea, a thiourea, a guanidyl, or a carbamoyl group, and at least one of R_1, R_3, R_4 and R_6 is a straight chain or branched, cyclic, alkyl, alkenyl, alkynyl or aryl group; Z, A_1, A_2 , m, n, i, j, k, 1, p, t and q are as defined above.

In the above formulae (D) and (E), R_1-R_6, Y, i, j, k, l, p, t and q are preferably as defined with reference to Formula (A).

It would be obvious for a skilled person that when Q is O or S, the number of substituents should be according their valency.

Certain of the compounds of the invention may be insufficiently soluble in physiological media to employ for delivery and transfection methods. Those of ordinary skill in the art will appreciate that there are a variety of techniques available in the art to enhance solubility of such compounds in aqueous media. Such methods are readily applicable without undue experimentation to the compounds described herein.

Definitions

Useful aryl groups are C_{6-100} aryl, preferably C_{6-75} aryl, more preferably C_{6-64} aryl, more preferably C_{6-50} aryl, more preferably C_{6-40} aryl, more preferably C_{6-30} aryl, most preferably C_{6-24} aryl. Typical C_{6-100} aryl groups include phenyl, naphthyl, phenanthryl, anthracyl, indenyl, azulenyl, biphenyl, biphenylenyl, fluorenyl, pyrenyl, aceanthrenyl, cholantrenyl, acephenanthrenyl, violantherenyl, hexaphenyl, hexacenyl, trinaphtyl and pyranthyl groups.

Useful alkyl groups are straight chain or branched C_{2-100} alkyl groups, preferably C_{4-75} alkyl, more preferably C_{6-64} alkyl, more preferably C_{8-50} alkyl, more preferably C_{8-40} alkyl, more preferably C_{8-30} alkyl, more preferably C_{6-30} alkyl, more preferably C_{4-30} alkyl, most preferably C_{8-24} alkyl. Typical C_{2-100} alkyl groups include ethyl, propyl, isopropyl, butyl, sec.-butyl, tert.-butyl, pentyl, hexyl, octyl, decyl, dodecyl, tetradecyl, hexadecyl, octadecyl, eicosyl, docosyl, tetracosyl, hexacosyl, octacosyl and triacontyl groups. Also contemplated is a trimethylene group substituted on two adjoining positions on any benzene ring of the compounds of the invention.

Useful alkenyl groups are straight chain or branched C_{2-100} alkenyl, preferably C_{4-75} alkenyl, more preferably C_{6-64} alkenyl, more preferably C_{8-50} alkenyl, more preferably C_{8-40} alkenyl, more preferably C_{8-30} alkenyl, more preferably C_{6-30} alkenyl, more preferably C_{4-30} alkenyl, most preferably C_{8-24} alkenyl. Typical C_{2-100} alkenyl groups include ethenyl, propenyl, isopropenyl, butenyl, sec.-butenyl, hexenyl, octenyl, decenyl, dodecenyl, especially

9-dodecenyl, tetradecenyl, especially 9-tetradecenyl, hexadecenyl, especially 9-hexadecenyl, octadecenyl, especially 9-octadecenyl, eicosenyl, docosenyl, tetracosenyl, hexacosenyl, octacosenyl and triacontenyl.

Useful alkynyl groups are straight chain or branched C_{2-100} alkynyl, preferably C_{4-75} alkynyl, more preferably C_{6-64} alkynyl, more preferably C_{8-50} alkynyl, more preferably C_{8-40} alkynyl, more preferably C_{8-30} alkynyl, more preferably C_{6-30} alkynyl, more preferably C_{4-30} alkynyl, most preferably C_{8-24} alkynyl. Typical C_{2-100} alkynyl groups include ethynyl, propynyl, butynyl, -butynyl, hexynyl, octynyl, decynyl, dodecynyl, tetradecynyl, hexadecynyl, octadecynyl, eicosynyl, docosynyl, tetracosynyl, hexacosynyl, octacosynyl and triacontynyl groups.

Typical alkyl ether groups include any of the above-mentioned C_{2-100} alkyl groups having an ether group.

An ether group is -O-.

Typical polyether groups include the -(CHR ^{14}-CH_2-O)t-, wherein R ^{14} is H or a C_{1-4} alkyl group and t is an integer as defined above, preferably t is 2 to 5.

For the purposes of the invention an amide group is an organic radical having -NHC(O)- as a functional group. Typical amide groups include alkyl amides, alkenyl amides, alkynyl amides, and aryl amides, wherein alkyl, alkenyl, alkynyl and aryl are as defined above.

Typically polyamide groups include organic radicals having two or more amide groups as defined above.

Typically an ester group is an organic radical having -C(O)-O- as a functional group. Typical ester groups include R ^{14}-C(O)-O-R ^{15}, wherein R ^{14} and R ^{15} are alkylene, alkenylene, alkynylene and arylene groups as defined above.

Typically urea groups are organic radicals having -NH-C(O)-NH- as a functional group. Typical urea groups include R ^{14}NH-C(O)-NHR ^{14}, R ^{14}NH-C(O)-NHR ^{15}, R ^{14}R ^{15}N-C(O)-

$NR^{14}R^{15}$ wherein R^{14} and R^{15} are alkylene, alkenylene, alkynylene and arylene groups as defined above.

Typically thiourea groups are organic radicals having urea group as defined above wherein the oxygen in the urea group is substituted by sulfur.

Typically guanidyl groups are organic radicals having -NH-C(NH)-NH- as a functional group. Typical guanidyl groups include R^{14}NH-C(NH)-NHR^{14}, R^{14}NH-C(NH)-NHR^{15} and R^{14} R^{15}N-C(NH)-N$R^{14}R^{15}$ wherein R^{14} and R^{15} are alkylene, alkenylene, alkynylene and arylene groups as defined above.

A carbamoyl group is -NH-C(O)-O-.

Typically carbonate groups include organic radicals containing a CO_3^{2-} radical, i.e., -O-C(O)-O.

A phosphate group is a PO_4^{3-} radical.

A sulfate group is a SO_4^{2-} radical.

A sulfoxide group is -S(O)-.

An imine group is -C(N}-.

A carbonyl group is -C(O)-.

A secondary amino group is -NH-.

Typically amino alcohol groups are organic radicals having both a secondary amino group as defined above and a hydroxyl group. Typical aminoalcohols include amino ethanol, aminopropanol and aminobutanol.

The definition "D is a bond" means that when D is not Q there is a single bond between $(CH_2)p$ and Z.

Biologically Active Substance refers to any molecule or mixture or complex of molecules that exerts a biological effect *in vitro* and/or *in vivo*, including pharmaceuticals, drugs, proteins, peptides, polypeptides, hormones, vitamins, steroids, polyanions, nucleosides, nucleotides, nucleic acids (e.g. DNA or RNA), nucleotides, polynucleotides, etc.

Cationic Lipids refers to any cationic lipids which may be used for transfection, including but not limited to, DOSPA, DOTMA, DMRIE, DOTAP, DOGS and TM -TPS.

Cell refers to eukaryotic cells of any type and from any source. Types of eukaryotic cells include epithelial, fibroblastic, neuronal, hematopoietic cells and the like from primary cells, tumor cells or immortalized cell lines. Sources of such cells include any animal such as human, canine, mouse, hamster, cat, bovine, porcine, monkey, ape, sheep, fish, insect, fungus and any plant including crop plants, ornamentals and trees.

Delivery is used to denote a process by which a desired compound is transferred to a target cell such that the desired compound is ultimately located inside the target cell or in, or on, the target cell membrane. In many uses of the compounds of the invention, the desired compound is not readily taken up by the target cell and delivery via lipid aggregates is a means for getting the desired compound into the cell. In certain uses, especially under in vivo conditions, delivery to a specific target cell type is preferable and can be facilitated by compounds of the invention.

Drug refers to any therapeutic or prophylactic agent other than food which is used in the prevention, diagnosis, alleviation, treatment, or cure of disease in man or animal.

Kit refers to transfection or protein expression kits which include one or more of the compounds of the present invention or mixtures thereof. Such kits may comprise a carrying means being compartmentalized to receive in close confinement one or more container means such as vials, test tubes and the like. Each of such container means comprises components or a mixture of components needed to perform transfection. Such kits may include one or more components selected from nucleic acids (preferably one or more vectors), cells, one or more compounds of the present invention, lipid-aggregate forming compounds, transfection enhancers, biologically active substances, etc.

Lipid Aggregate is a generic term which includes liposomes of all types both unilamellar and multilameller as well as micelles and more amorphous aggregates of cationic lipids or lipids mixed with amphiphatic lipids such as phospholipids and steroids.

Lipid Aggregate-forming Compounds refers to neutral compounds or lipids such as DOPE, DOPC and cholesterol, etc.

Target Cell refers to any cell to which a desired compound is delivered, using a lipid aggregate as carrier for the desired compound.

Transfection is used herein to mean the delivery of nucleic acid, protein or other macromolecule to a target cell, such that the nucleic acid, protein or other macromolecule is expressed or has a biological function in the cell. The term "expressible nucleic acid" includes both DNA and RNA without regard to molecular weight, and the term "expression" means any manifestation of the functional presence of the nucleic acid within the cell including, without limitation, both transient expression and stable expression. Functional aspects include inhibition of expression by oligonucleotides or protein delivery.

Transfection Enhancers refers generally to molecules and substances such as proteins, peptides, growth factors and the like that enhance cell-targeting, uptake, internalization, nuclear targeting and expression. Such molecules and substances include ligands such as insulin, transferrin, fibronectin that target the cell surface; peptides that target cellular integrin receptors; and other compounds such as Plus Reagent (available from Life Technologies, Inc., Rockville, Maryland). Examples of transfection enhancers may be found in U.S. Patent No.5, 736,392 and U.S. application Serial No. 09/039,780 filed March 16, 1998.

The invention will be further clarified by the following examples, which are intended to be purely exemplary of the invention. The polycationic lipids were prepared by following the general reaction schemes described below.

EXAMPLES

Example 1

Synthesis of N^1,N^4-dioleoyl-diam in obutane (I)

A solution of 1,4-diaminobutane (4.28 g, 48.6 mmol) and triethylamine (20.4 ml, 146 mmol) in 10 mL of dry methylene chloride was slowly added to a solution of oleoyl chloride (30.0 g, 99.7 mmol) in 300 ml of anhydrous methylene chloride in an ice bath 25 at 0°C. The reaction mixture was stirred vigorously with a mechanical stirrer. After the addition was complete, the ice bath was removed and the mixture was stirred at room temperature for 2.5 days. TLC analysis confirmed that the reaction had gone to completion and the product had precipitated. The excess oleoyl chloride was removed by filtration. The

precipitate was washed twice with 50 ml of methylene chloride. The mother liquor was concentrated and more product precipitated. This precipitate was filtered and combined with the previous precipitate. The resulting solid was vacuum dried for 4 hours. A total of 27.0 g of a white solid of the desired product, N^1,N^4-dioleoyl-diaminobutane, was obtained.

Synthesis of N1,N -dioleyl-diaminobutane (II)

Lithium aluminum hydride (8.62 g, 95%, 216 mmol) was carefully added to a suspension of N^1,N^4-dioleoyl-diaminobutane (27.0 g, 43.8 mmol) in 400 ml of anhydrous diethyl ether at 0°C. After addition, the ice bath was removed. The reaction mixture was warmed slowly to room temperature and then heated gently to reflux with an appropriate condensing device and stirred for 16 hours. The reaction mixture was then cooled and quenched carefully at 0°C with 70 mL of a 1 N sodium hydroxide solution. Another 500mL of diethyl ether was added and the mixture was stirred at room temperature for additional 2 hours. The top ether layer turned clear gradually and then separated. The aqueous layer was extracted three times with 100 mL of diethyl ether each. The combined ether solution was concentrated, and dried on high vacuum overnight. Total of 17.0 g of oily colorless N^1,N^4-dioleyl-diaminobutane was obtained.

Synthesis of N^1,N^4-dioleyl-N^1,N^4 -di-[2-hydroxy-3-(N -phthalamido) propyljdiamino-butane (III)

Diisopropylethylamine (11.1 mL, 63.7 mmol) was added to a suspension of N^1,N^4-dioleyl-diaminobutane (15.5 g, 26.3 mmol) and N-(2,3-epoxypropyl)-phthalimide (15.6g, 76.8 mmol) in 110 mL of dry N,N-dimethylformamide. After purging with nitrogen, the reaction mixture was sealed in a round-bottom flask and heated to around 90°C for 24 hours. N,N-dimethylformamide and diisopropylethylamine were removed and a yellow oil was obtained. This crude material was recrystallized from ethanol. A total of 18.6 g of a white solid, N^1,N^4-dioleyl-N^1,N^4-di-[2-hydroxy-3-(N-phthalamido) propyl]-diamino-butane was obtained.

Synthesis of N^1,N^4 -dioleyl-N^1,N^4-di-[2-hydroxy-3-(N-aminopropyl)]-diaminobutane (IV) (hereinafter referred to as DHDOS)

Hydrazine (4.0 mL, 80% aq., 103 mmol) was added to a suspension of N^1,N^4-dioleyl- N^1,N^4-di-[2-hydroxy-3-(N-phthalamido)propyl]-diaminobutane (17.0 g, 17.1

mmol) in 250 mL of dry ethanol at room temperature. With an appropriate condensing device, the reaction mixture was heated to a reflux, at which point the suspension turned into a clear solution. The oil bath was set to 85°C. After 45 minutes a white solid precipitated from the solution. The reaction mixture was stirred at reflux for 4 hours before being cooled to -20°C. The white solid settled down to the bottom. The top clear ethanol solution was decanted. The residue was washed twice with cold ethanol. The combined ethanol solution was concentrated and dried overnight over vacuum. 12.4g of oily N^1,N^4-dioleyl-N^1,N^4-di-[2-hydroxy-3-(N-aminopropyl)]-diaminobutane was obtained.

The following compounds were synthesized by the above method using the corresponding diamine and a long chain acyl chloride:

N^1,N^4-dimyristyl-N^1,N^4-di-[2-hydroxy-3-(N-aminopropyl)]-diaminobutane;
N^1,N^4-dipalmityl-N^1,N^4-di-[2-hydroxy-3-(N-aminopropyl)]-diaminobutane;
N^1,N^4-dipalmitolyl-N^1,N^4-di-[2-hydroxy-3-(N-aminopropyl)]-diaminobutane;
N^1,N^4-distearyl-N^1,N^4-di-[2-hydroxy-3-(N-aminopropyl)]-diaminobutane;
N^1,N^4-dilauryl-N^1,N^4-di-[2-hydroxy-3-(N-aminopropyl)]-diaminobutane;
N^1,N^2-dimyristyl-N^1,N^2-di-[2-hydroxy-3-(N-aminopropyl)]-diaminoethane;
N^1,N^2-dipalmity-N^1,N^2-di-[2-hydroxy-3-(N-aminopropyl)]-diaminoethane;
N^1,N^2-dipalmitolyl-N^1,N^2-di-[2-hydroxy-3-(N-aminopropyl)]-diaminoethane;
N^1,N^2-distearyl-N^1,N^2-di-[2-hydroxy-3-(N-aminopropyl)]-diaminoethane;
N^1,N^2-dilauryl-N^1,N^2-di-[2-hydroxy-3-(N-aminopropyl)]-diaminoethane;
N^1,N^2-dioleyl-N^1,N^2-di-[2-hydroxy-3-(N-aminopropyl)]-diaminoethane;
N^1,N^8-dimyristyl-N^1,N^8-di-[2-hydroxy-3-(N-aminopropyl)]-Jeffamine;
N^1,N^8-dipalmityl-N^1,N^8-di-[2-hydroxy-3-(N-aminopropyl)]-Jeffamine;
N^1,N^8-dipalmitolyl-N^1,N^8-di-[2-hydroxy-3-(N-aminopropyl)]-Jeffamine;
N^1,N^8-distearyl-N^1,N^8-di-[2-hydroxy-3-(N-aminopropyl)]-Jeffamine;
N^1,N^8-dilauryl-N^1,N^8-di-[2-hydroxy-3-(N-aminopropyl)]-Jeffamine;
N^1,N^8-dioleyl-N^1,N^8-di-[2-hydroxy-3-(N-aminopropyl)]-Jeffamine;

Synthesis of N^1,N^4-dioleyl- N^1,N^4-di-[2-hydroxy-3-(N-carboxamidine)- aminopropyl]-diaminobutane (V)

1H-pyrazole-1-carboxamidine hydrochloride (45 mg, 0.31 mmol) was added to a solution of N^1,N^4-dioleyl-N^1,N^4-di-[2-hydroxy-3-(N-aminopropyl)]-diamino-butane

(115 mg, 0.156 mmol) in 1 mL of dry N,N-dimethylformamide. The salt was not very soluble in dimethylformamide (DMF). However, the mixture turned clear after diisopropylethylamine (55 µl, 0.31 mmol) was added. The mixture was stirred under nitrogen at room temperature for 18 hours. After removal of solvent, the crude material was loaded on a C-18 reverse phase flash column, and eluted with 20% H2O in MeOH to 10% H2O in MeOH. The pure fractions were collected and concentrated. An 81 mg colorless oily N^1,N^4-dioleyl-N^1 ,N^4-di -[2-hydroxy-3-(N -carboxamidine)aminopropyl]-diaminobutane was obtained, which was converted to its TFA and HCL salts.

Synthesis of N^1,N^4-dioleyl-N^1,N^4-di-{2-hydroxy-3-[N(N^I,N^{II},N^{III},N^{IV}-butoxycarbonyl-spermine carboxamido)] aminopropyl}diaminobutane (VI)

Diisopropylcarbodiimide (5.32 mL, 34.0 mmol) was added drop wise to a solution of Boc-spermine acid (21.7 g, 33.5 mmol) and N-hydroxysuccinimide (NHS) (3.91 g, 34.0 mmol) in mixed solvents (100 mL of DMF and 100 mL of CH_2Cl_2) at room temperature. After stirring for 2.5 hours, a solution of N^1,N^4-dioleyl-N^1,N^4-di-[2-hydroxy-3-(N-aminopropyl)] diaminobutane (10 g, 13.6 mmol) in 40 mL of methylene chloride and DMF was added. The mixture was stirred for another 5 hours before quenching with 200 mL of a 2.5% sodium bicarbonate solution. An additional 300 mL of methylene chloride was added. The aqueous solution was extracted with 120 mL of methylene chloride three times. The combined organic solution was washed with water twice and dried over anhydrous magnesium sulfate. After concentration, a pale yellow oil was obtained. The crude material was purified with silica gel, eluting with 2% MeOH in CH_2Cl_2 to 5% MeOH in CH_2Cl_2. A total of 13.1 g of white solid N^1,N^4-dioleyl- N^1,N^4- di-{2-hydroxy-3-[N-(N^I,N^{II}, ,N^{III} ,N^{IV}-butoxycarbonyl-spermine carboxamido)] aminopropyl}di-aminobutane was obtained.

Synthesis of N^1,N^4-dioleyl-N^1,N^4-di-[2-hydroxy-3-(N-spermine carboxamido)-aminopropyl] -diaminobutane (VII)

100 mL of a solution of 4.0 M hydrogen chloride in 1,4-dioxane was added to a solution of N^1,N^4-dioleyl-N^1,N^4-di-{2-hydroxy-3-[N-(N^I,N^{II},N^{III},N^{IV} butoxycarbonylspermine carboxamido)] aminopropyl}diaminobutane (11.8g, 5.92 mmol) in l00mL of l,4-dioxane at room temperature. The reaction mixture turned cloudy 10 minutes after addition of the acid. After 2.5 hours of stirring at room temperature, the excess acid and solvent was removed. The residue was dried for at least 5 hours over vacuum before being loaded on a C-18 reverse phase flash column. The column was eluted starting with 25% H_2O in MeOH, then 20%, and then 17%. Pure fractions were collected and concentrated. A 3.06 g colorless solid N^1,N^4-

dioleyl-N^1,N^4-di-[2-hydroxy-3-(N-spenninecarboxamido)-aminopropyl]-diaminobutane was obtained.

The following compounds were synthesized using the protocol described above starting with the requisite diamine and long chain acyl chloride:

N^1,N^4-dimyristyl-N^1,N^4-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-diaminobutane;

N^1,N^4-dipalmityl-N^1,N^4-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-diaminobutane;

N^1,N^4-dipalmitolyl-N^1,N^4-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-diaminobutane;

N^1,N^4-distearyl-N^1,N^4-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-diaminobutane;

N^1,N^4-dilauryl-N^1,N^4-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-diaminobutane;

N^1,N^8-dimyristyl-N^1,N^8-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-Jeffamine;

N^1,N^8-dipalmityl-N^1,N^8-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-Jeffamine;

N^1,N^8-dipalmitolyl-N^1,N^8-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-Jeffamine;

N^1,N^8-distearyl-N^1,N^8-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-Jeffamine;

N^1,N^8-dilauryl-N^1,N^8-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-Jeffamine;

N^1,N^8-dioleyl-N^1,N^8-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-Jeffamine;

N^1,N^2-dimyristyl-N^1,N^2-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-diaminoethane;

N^1,N^2-dipalmityl-N^1,N^2-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-diaminoethane;

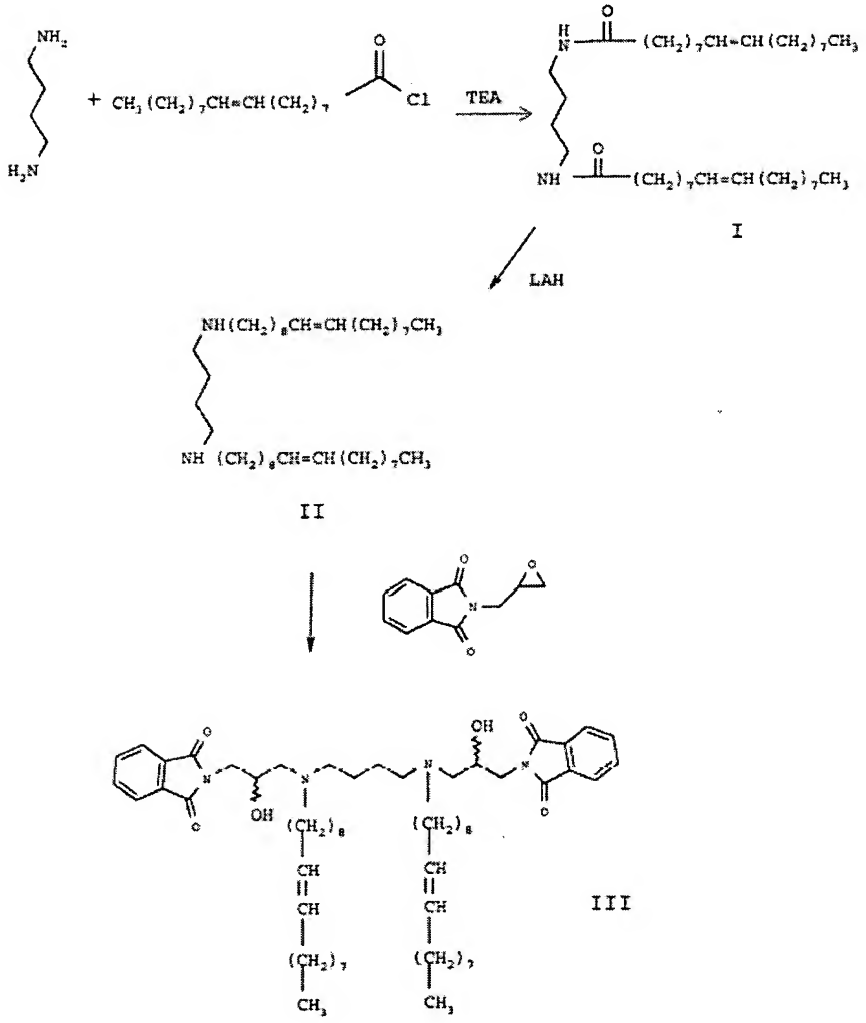
N^1,N^2-dipalmitolyl-N^1,N^2-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-diaminoethane;

N^1,N^2-distearyl-N^1,N^2-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-diaminoethane;

N^1,N^2-dilauryl-N^1,N^2-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-diaminoethane;

N^1,N^2-dioleyl-N^1,N^2-di-[2-hydroxy-3-(N-sperminecarboxamido)-aminopropyl]-diaminoethane;

Synthesis of dihydroxy-dioleyol-disperminecarboxamido spermine and analogs (Scheme 1)

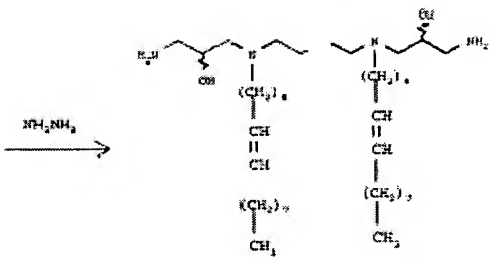


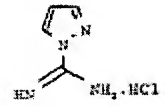
I

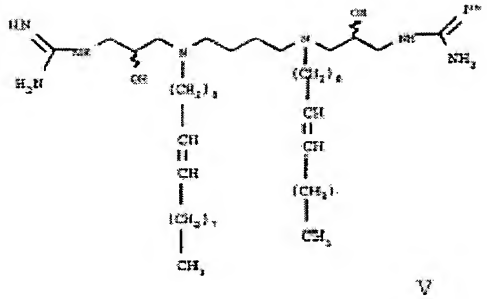
II

III

IV (DHDOS)

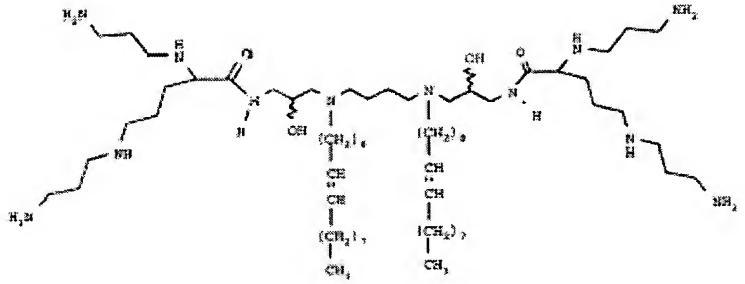






V

1 Boc-spermic acid (VI)
2) acid



VII

Example 2

Synthesis of N^1,N^4-dioleyl-N^1,N^4-di-3-cyanopropyldiaminobutane (VIII)

Acrylonitrile (0.43 mL, 6.53 mmol) was added dropwise to a solution of N^1,N^4-dioleyl-diaminobutane (1.8 g, 3.06 mmol) in 20 mL of ethanol at room temperature. The mixture was stirred for 30 hours. All starting materials were converted to product as confirmed by TLC analysis. The crude material was purified using flash chromatography with a silica gel (1 % MeOH in CH_2Cl_2. A clear oil was obtained at high yield.

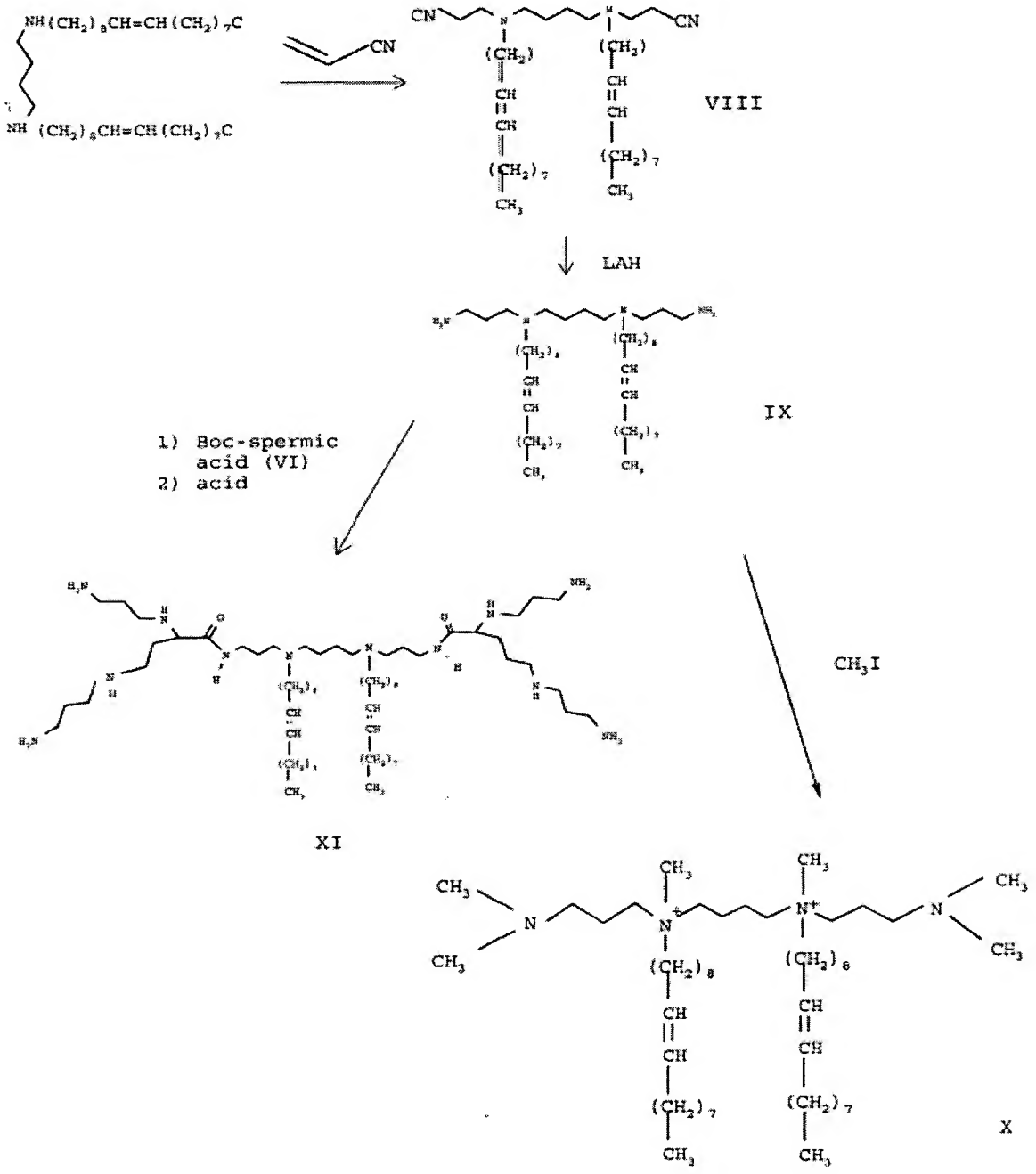
Synthesis of N^1,N^4-dioleyl-N^1,N^4-di-3-(aminopropyl)-diaminobutane (IX)

A solution of LAH (9.2 mL, 1 M in ether, 9.2 mmol) was slowly added to a solution of N^1,N^4-dioleyl-N^1,N^4-di-3-cyanopropyl-diaminobutane (2.12 g, 3.05 mmol) in 15 mL of anhydrous diethyl ether at 0°C. After addition, the mixture was stirred at room temperature for 20 hours. All starting material was consumed. The reaction mixture was quenched with a 1 N NaOH solution at 0°C. After stirring 2 hours at room temperature, the mixture was extracted with diethyl ether three times. The combined ether solutions were concentrated and dried over vacuum for three hours. An oily N^1,N^4-dioleyl-N^1,N^4- di-3-(aminopropyl)diaminobutane was obtained at high yield.

Synthesis of N^1,N^4-dioleyl-N^1,N^4-di-[3-(N-spermine carboxamido)-aminopropyl]-diaminobutane (XI)

The procedure for making N^1,N^4-dioleyl-N^1,N^4-di-[2-hydroxy-3-(N-spermine carboxamido)-aminopropyl]-diaminobutane described above was followed.

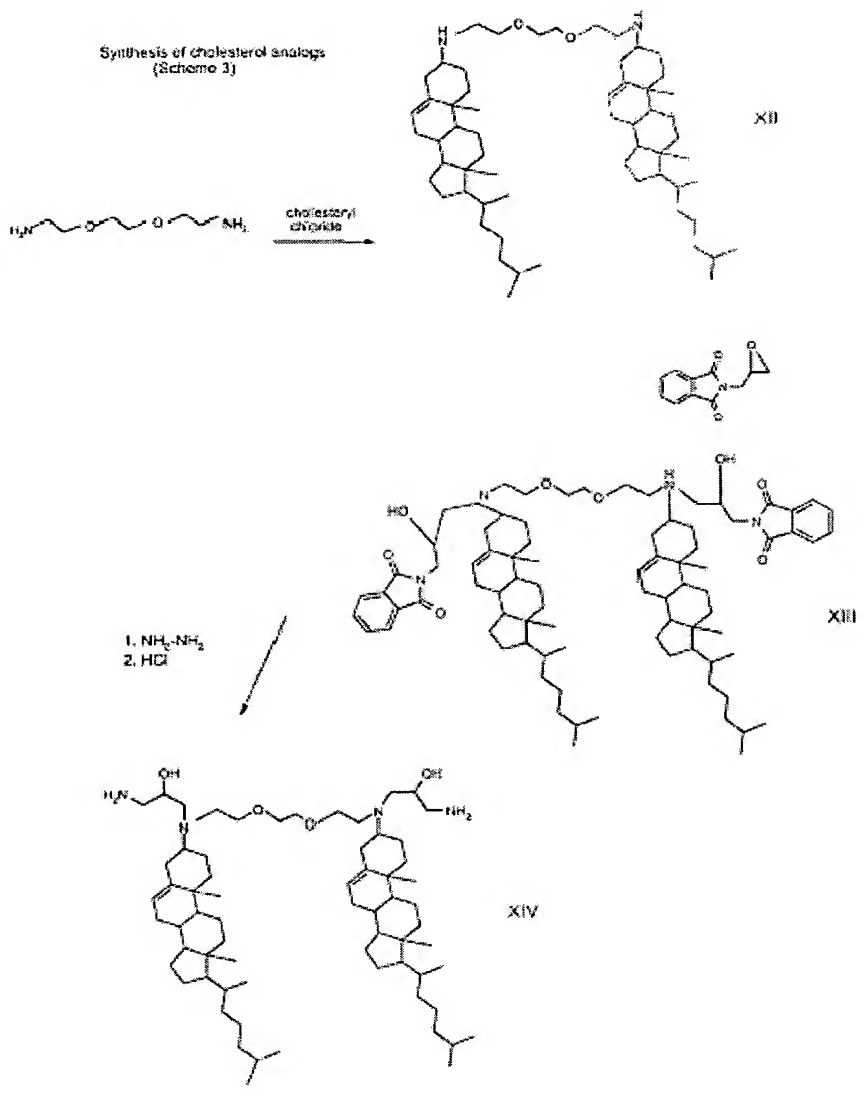
Synthesis of dioleyol-disperminecarboxamido spermine and analogs (Scheme 2)



Example 3

Synthesis of cholesterol analogs

The cholesterol analogs can be synthesized by using the scheme given below (Scheme 3). Jeffamine is alkylated with cholestryl chloride to provide the dicholestryl jeffamine analog (XII). Further alkylation with the epoxide phthalamide (XIII) and deblocking with hydrazine gives the compound of the invention (XIV).

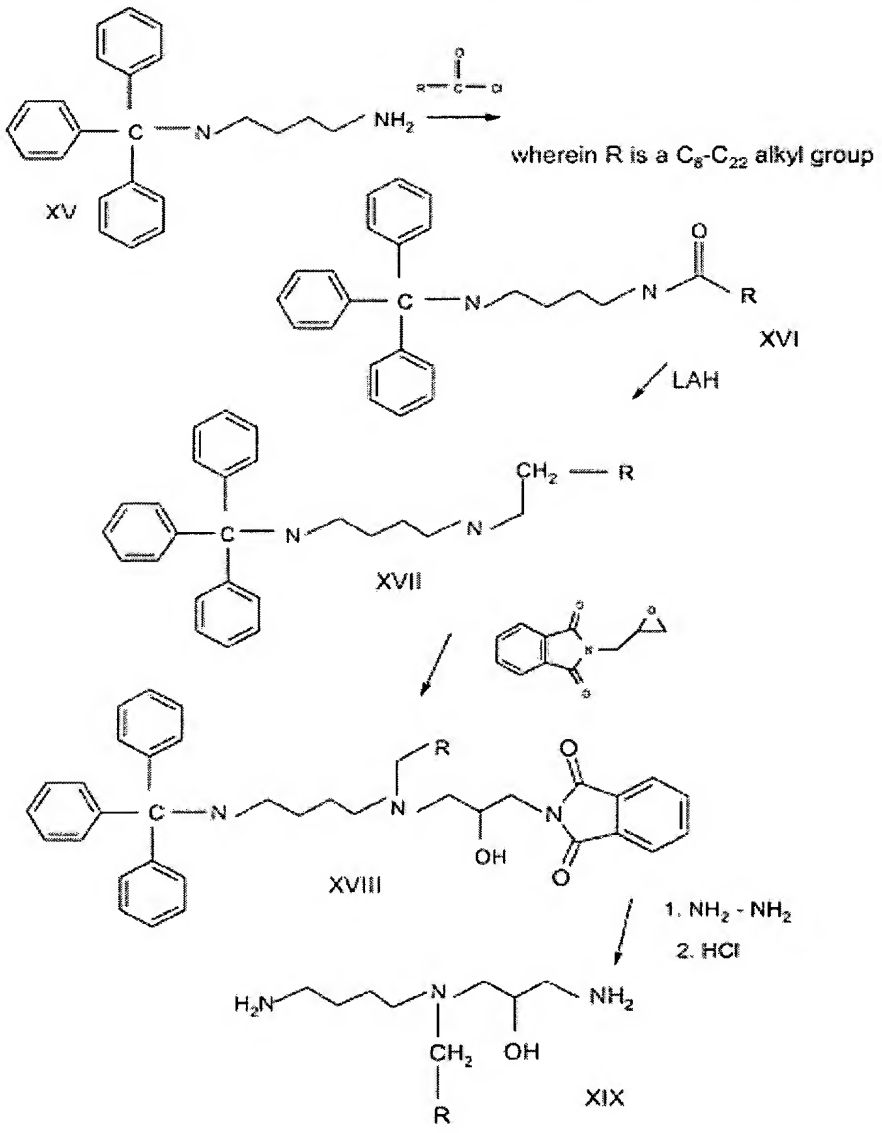

Synthesis of cholesterol analogs
(Scheme 3)
cholesteryl chloride
XII
XIII
1. NH2-NH2
2. HCl
XIV

Example 4

Synthesis of monoalkyl analogs

When monoalkyl analogs are desired, the above Scheme 1 can be modified such that one of the amines in the starting material is protected before the acylation step. Thus, tritylprotected diaminobutane (XV) is acylated with alkanoyl chloride (e.g., oleoyl chloride) followed with LAH reduction to obtain compound XVIII. The amine is then alkylated with the desired phthalamide epoxide to obtain compound XVIII. Removing the phthalamide using hydrazine renders the desired amine XIX. (See Scheme 4).

Synthesis of monoalkyl analogs (Scheme 4):

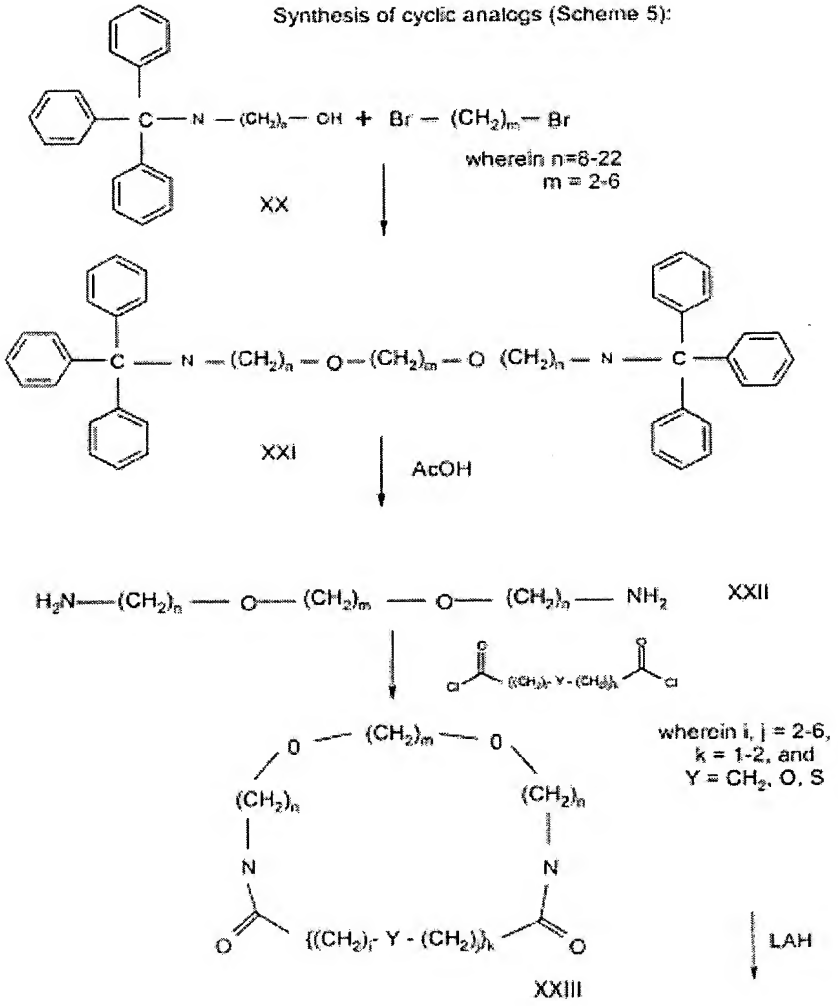


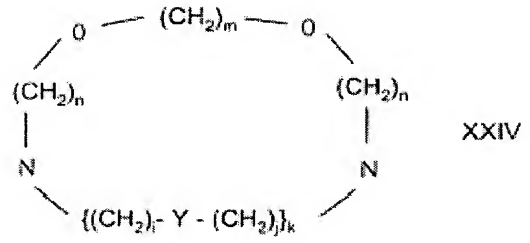
Example 5

Synthesis of cyclic analogs

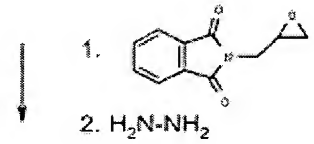
The following scheme (Scheme 5) can be used to make the cyclic analogs. Trityl protected amino alcohol (XX) with the desired chain is alkylated using dibromoalkyl (e.g., dibromobutane). The trityl is removed from the desired dimer (XXI) and acylated using diacyl chlorides (e.g., succinyl chloride). The amide (XXIII) is then reduced with LAB and alkylated using the desired phthalamide epoxide. Removal of the phthalamide gives the desired compound of the invention.

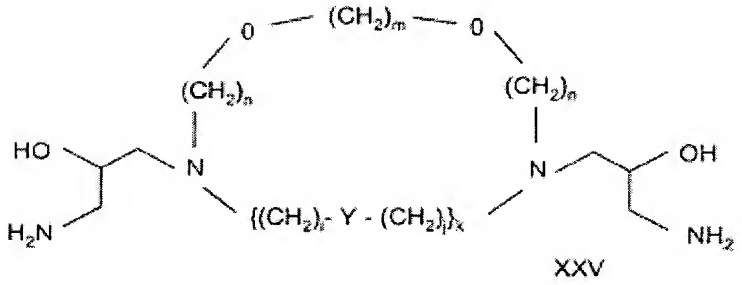
Synthesis of cyclic analogs (Scheme 5):




O
(CH2)m
O
(CH2)n
(CH2)n
N
N
{(CH2)i- Y - (CH2)j}k

XXIV


1.
2. H2N-NH2


O
(CH2)m
O
(CH2)n
(CH2)n
HO
N
N
OH
H2N
{(CH2)i- Y - (CH2)j}k
NH2

XXV

Example 6

Synthesis of polymeric analogs

Polymeric analogs of the present invention can be synthesized by using polymeric amines such as PEI as starting material or dendrimeric polyamines. For example, PEI can be acylated with alkyloyl chloride (e.g., oleoyl chloride) and the acylated PEI can then be reduced with lithium aluminum hydride to obtain compounds of the invention.

Although the above methods exemplify the synthesis of specific compounds, the reaction schemes provide a general method for preparing a variety of compounds according to the present invention. Those of ordinary skill in the art will appreciate that alternate methods and reagents other than those specifically detailed herein can be employed or readily adapted to produce compounds of the invention.

The compounds of the present invention can be used in the same manner as are prior art compounds such as DOTMA, DOTAP, DOGS, DOSPA and the like. Methods for incorporating such cationic lipids into lipid aggregates are well-known in the art. Representative methods are disclosed by Felgner, et al., supra; Eppstein et al. supra; Behr et al. supra; Bangham, A. et al. (1965) M. Mol. Biol. 23:238-252; Olson, F. et al. (1979) Biochim. Biophys. Acta 557:9-23; Szoka, F. et al. (1978) Proc. Natl. Acad. Sci. USA 75:4194-4198; Mayhew, E. et al. (1984) Biochim. Biophys. Acta 775:169-175; Kim, S. et al. (1983) Biochim. Biophys. Acta 728: 339-348; and Fukunaga, M. et al. (1984) Endocrinol. 115:757-761. Techniques for preparing lipid aggregates of appropriate size for use as delivery vehicles include sonication and freeze-thaw plus extrusion as perhaps the most commonly used. See, e.g., Mayer, L. et al. (1986) Biochim. Biophys. Acta 858:161-168. Microfluidization is used when consistently small (50-200 nm) and relatively uniform aggregates are desired (Mayhew, E., supra). Aggregates ranging from about 50 nm to about 200 nm diameter are preferred; however, both larger and smaller sized aggregates are functional.

Methods of transfection and delivery of other compounds are well-known in the art. The compounds and compositions of the present invention yield lipid aggregates that can be used in the same processes used for other known transfection agents.

It will be readily apparent to those of ordinary skill in the art that a number of general parameters are important for optimal efficiency of transfection or delivery. These parameters include, for example, the cationic lipid concentration, the concentration of compound to be delivered, the number of cells transfected, the medium employed for delivery, the length of time the cells are incubated with the polyanion-lipid complex, and the relative amounts of cationic and non-cationic lipid. It may be necessary to optimize these parameters for each particular cell type. Such optimization is routine employing the guidance provided herein and knowledge generally available to the art.

It will also be apparent to those of ordinary skill in the art that alternative methods, reagents, procedures and techniques other than those specifically detailed herein can be employed or readily adapted to produce the liposomal precursors and transfection compositions of this invention. Such alternative methods, reagents, procedures and techniques are within the spirit and scope of this invention.

The use of representative compounds of the invention are further detailed by reference to the following examples. All abbreviations used herein are standard abbreviations in the art. Specific procedures not described in detail are either referenced or well-known in the art.

Example 7

This example compares transfection of HEK-293 (human embryonic kidney-derived cell line), COS-7 (SV40 transformed monkey cell line), CHO-KI (Chinese Hamster Ovary-derived cell line), and HeLa (Human cervical carcinoma-derived cell line) cells with the β-galactosidase reporter plasmid DNA pCMV•SPORT-β-gal (LifeTechnologies, Rockville, MD) using commercially available cationic lipid transfection reagents and the compounds of the present invention.

The cells were plated the day before transfection in 24-well tissue culture plates in a total volume of 0.4ml DMEM (Dulbecco's Modified Eagle's Medium, Life Technologies, Rockville, MD) culture medium containing a 1 % non-essential amino acid (NEAA) solution (LifeTechnologies), and 10%FBS. For the HEK-293 andCOS-7 cells, tissue culture plates were pre-coated with Poly-L-Lysine to enhance cell attachment.

The next day, DNA-transfection reagent complexes were prepared as follows:

The cationic lipid reagents and DNA were diluted separately into 25 µl aliquots of serum-free DMEM, containing 1 % NEAA. For LipofectAMINE PLUS, 7-14 µl of PLUS reagent was added to the DNA, mixed, and incubated for 15 minutes at room temperature. The diluted DNA was combined with the diluted lipid and incubated at room temperature for at least 15 minutes to allow the DNA and the lipid to form complexes. Following this incubation the complexes were added directly into the culture medium dropwise and mixed by rocking the culture plate back and forth. The cells were further incubated at 37°C for a total of 24 hours to allow expression of the lacZ transgene encoded by the reporter plasmid, pCMV•SPORT-β-gal. At 24 hours post-transfection, the growth medium and transfection complexes were removed from the wells, and the cells in each well were rinsed briefly with 1ml of D-PBS (Dulbecco's PBS, Life Technologies, Rockville, MD). The cells in each well were lysed by the addition of 0.15 to 2.0 ml of 0.1 % Tris, pH 8.0, containing 0.1 M Triton X-100. The plates were frozen at -80°C for a minimum of 2 hours, and thawed at room temperature or 37°C. The thawed cell lysates were cleared by centrifugation and the supernatants were assayed for β-gal activity using the enzymatic substrate ONPG. The concentration of total protein in cell lysates was also determined using a Bradford assay (Bio-Rad Laboratories,Hercules CA). β-gal activity in transfected cell extracts was calculated against a standard curve and expressed as ng β-gal per surface area of tissue culture plate (ng/cm2) to reflect activity per transfection, or as ng β-gal per µg of total protein (ng/µg) to reflect specific activity.

HEK-293 (Figure 1), COS- 7 (Figure 2), CHO-K1 (Figure 3), and HeLa (Figure 4) cells were transfected with 0.4 or 0.8 µg of pCMV•SPORT-β-gal DNA and 0.2 to 4.0 µl of transfection reagent. The transfection reagents tested were DHDOS (IV) formulated at 2mg/ml with the neutral co-lipid, cholesterol (at a ratio of 1:15 (M/M) DHDOS to cholesterol); DHDOS formulated at 2mg/ml with the neutral co-lipid DOPE (dioleylphosphatidyl ethanolamine) (at a ratio of 1:1 (M/M) DHDOS to DOPE); LipofectAMINE PLUS (Life Technologies, Rockville MD); and FuGENE^{TM}-6 (Boehringer Mannheim,Germany). DHDOS formulations were tested in the range of 0.2 to 1.5 µl; LipofectAMINE PLUS and FuGENE-6 were tested in the range of 0.2 to 4.0 µl. FuGENE-6 was used according to the manufacturer's recommended protocol. DHDOS and LipofectAMINE PLUS were used according to the above protocol. The data presented in the Figures are expressed as total activity (ng/cm^2) to better compare total expression from the

transfected DNA. Only data with 0.8 μg of DNA is shown, since similar results were obtained with 0.4 and 0.8 μg of DNA.

Example 8

Primary, passaged, normal human fibroblasts (NHFs) were plated in 96-well plates at a density of 1.6 X 104 cells per well and transfected the following day. Cells in each well were transfected with 40 ng pCMV•SPORT-β-gal DNA and 0.1 or 0.2 μl lipid. The DNA and lipid were diluted separately into 10 μl of DMEM. The DNA was either used alone or pre-mixed with PLUS, insulin, transferrin, or an integrin-targeting peptide prior to complexing with the lipid. After 15 minutes of complexing, the DNA-lipid was added to cells. Cells were assayed for p-gal activity as described above.

ACTIVITY (ng/βgal/cm²)

LIPID	DNA	DNA and PLUS*	DNA and INSULIN	DNA and TRANSFERRIN	DNA and INTEGRIN-TARGETING PEPTIDE**
LipofectAMINE	10.36	28.6	ND	17.4	ND
Compound of Formula X 1:1.5 DOPE 1 mg/ml	ND	37.8	ND	ND	40.9
Compound of Formula VII 1:1 DOPE 2 mg/ml	29.4	637.9	195.7	21.7	587.9

ND = no detectable activity
* PLUS Reagent is available from Life Technologies, Inc., Rockville, Maryland.
** Reference: S.L. HART, et al (1998). Human Gene Therapy, 9:575-585.

The results show that these cationic lipid formulations can deliver DNA molecules alone, but also that delivery, and ultimately gene expression, may be enhanced when the lipids are used in conjunction with peptides or proteins that bind DNA and/or act as ligands for cell surface receptors, or otherwise enhance cellular and/or nuclear uptake.

Having now fully described the present invention in some detail by way of illustration and examples for purposes of clarity of understanding, it will be obvious to one of ordinary skill in the art that the same can be performed by modifying or changing the invention within

a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or any specific embodiment thereof, and that such modifications or changes are intended to be encompassed within the scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains, and are herein incorporated by reference to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference.